US006093809A

United States Patent [19]

Cech et al.

[11] Patent Number: 6,093,809

[45] Date of Patent: Jul. 25, 2000

[54] TELOMERASE

[75] Inventors: Thomas R. Cech, Boulder, Colo.; Joachim Lingner, Epalinges, Switzerland

[73] Assignees: University Technology Corporation, Boulder, Colo.; Geron Corporation, Menlo Park, Calif.

[21] Appl. No.: 08/851,843

[22] Filed: May 6, 1997

Related U.S. Application Data

[63] Continuation-in-part of application No. 08/846,017, Apr. 25, 1997, which is a continuation-in-part of application No. 08/844,419, Apr. 18, 1997, which is a continuation-in-part of application No. 08/724,643, Oct. 1, 1996.

[51] Int. Cl.[7] C07H 21/04; A61K 38/00; C07K 5/00; C07K 7/00

[52] U.S. Cl. 536/23.5; 536/23.2; 530/324

[58] Field of Search 536/23.1, 23.2, 536/23.5; 530/324

[56] References Cited

U.S. PATENT DOCUMENTS

3,817,837	6/1974	Tanenholtz et al.	195/103.5
3,850,752	11/1974	Schuurs et al.	195/103.5
3,939,350	2/1976	Kronick et al.	250/365
3,996,345	12/1976	Ullman et al.	424/12
4,275,149	6/1981	Litman et al.	435/7
4,277,437	7/1981	Maggio	422/61
4,366,241	12/1982	Tom et al.	435/7
4,683,195	7/1987	Mullis et al.	435/6
4,683,202	7/1987	Mullis	435/91
4,816,567	3/1989	Cabilly et al.	530/387
4,965,188	10/1990	Mullis et al.	435/6
5,489,508	2/1996	West et al.	435/6
5,583,016	12/1996	Villeponteau et al.	435/91.3

FOREIGN PATENT DOCUMENTS

09154575	6/1997	Japan	A61K 38/51
WO 84/03564	9/1984	WIPO	G01N 33/54
WO 96/12811	5/1996	WIPO	C12N 15/54
WO 96/19580	6/1996	WIPO	C12N 15/54
WO 96/40869	12/1996	WIPO	C12N 5/00
WO 98/01542	1/1998	WIPO	C12N 9/12
WO 98/01543	1/1998	WIPO	C12N 9/12
WO 98/45450	10/1998	WIPO	C12N 15/54

OTHER PUBLICATIONS

Zakian, "Telomeres: Beginning to Understand the End," *Science* 270:1601 [1995].

Blackburn and Gall, "A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena," *J. Mol. Biol.*, 120:33 [1978].

Oka et al., "Inverted terminal repeat sequence in the macronuclear DNA of *Stylonychia pustulata*," *Gene* 10:301 [1980].

Klobutcher et al., "All gene-sized DNA molecules in four species of hypotrichs have the same terminal sequence and an unusual 3' terminus," *Proc. Natl. Acad. Sci.*, 78:3015 [1981].

Lingner et al., "Telomerase RNAs of different ciliates have a common secondary structure and a permuted template," *Genes Develop.*, 8:1984 [1994].

Biessmann et al., "Addition of Telomere-Associated HeT DNA Sequences "Heals" Broken Chromosome Ends in Drosophilia," *Cell* 61:663 [1990].

Sheen and Levis, "Transposition of the LINE-like retrotransposon TART to Drosophilia chromosome termini," *Proc. Natl. Acad. Sci.*, 91:12510 [1994].

Kipling and Cooke, "Hypervariable ultra-long telomeres in mice," *Nature* 347:400 [1990].

Starling et al., "Extensive telomere repeat arrays in mouse are hypervariable," *Nucleic Acids Res.*, 18:6881 [1990].

Shampay and Blackburn, "Generation of telomere-length heterogeneity in *Saccharomyces cerevisiae*," *Proc. Natl. Acad. Sci.*, 85:534 [1988].

Lustig and Petes, "Identification of yeast mutants with altered telomere structure," *Proc. Natl. Acad. Sci.*, 83:1398 [1986].

Sandell et al., "Transcription of yeast telomere alleviates telomere position effect without affecting chromosome stability," *Proc. Natl. Acad. Sci.*, 91:12061 [1994].

Chan and Tye, "Organization of DNA sequences and replication origins at yeast telomeres," *Cell* 33:563 [1983].

Wright et al., "Saccharomyces telomeres assume a non-nucleosomal chromatin structure," *Genes Develop.*, 6:197 [1992].

Gottschling and Cech, "Chromatin Structure of the Molecular Ends of Oxytricha Macronuclear DNA: Phased Nucleosomes and a Telomeric Complex," *Cell* 38:501 [1984].

Blackburn and Chiou, "Non-nucleosomal packaging of a tandemly repeated DNA sequence at termini of extrachromosomal DNA coding for rRNA in Tetrahymena," *Proc. Natl. Acad. Sci.*, 78:2263 [1981].

Braunstein et al., "Transcriptional silencing in yeast is associated with reduced nucleosome acetylation," *Genes Develop.*, 7:592 [1993].

Makarov et al., "Nucleosomal Organization of Telomere-Specific Chromatin in Rat," *Cell* 73:775 [1993].

Tommerup et al., "Unusual chromatin in human telomeres," *Mol. Cell. Biol.*, 14:5777 [1994].

(List continued on next page.)

Primary Examiner—Yvonne Eyler

Attorney, Agent, or Firm—Townsend and Townsend and Crew LLP

[57] ABSTRACT

The present invention is directed to novel telomerase nucleic acids and amino acids. In particular, the present invention is directed to nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs, including the 123 kDa and 43 kDa telomerase protein subunits of Euplotes aediculatus, and related sequences from Schizosaccharomyces, Saccharomyces sequences, and human telomerase. The present invention is also directed to polypeptides comprising these telomerase protein subunits, as well as functional polypeptides and ribonucleoproteins that contain these subunits.

1 Claim, 71 Drawing Sheets

-continued

```
Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

What is claimed is:

1. An isolated polynucleotide consisting of the nucleic acid sequence shown in SEQ. ID. No. 1.

* * * * *

US006166178A

United States Patent [19]

Cech et al.

[11] Patent Number: 6,166,178

[45] Date of Patent: Dec. 26, 2000

[54] TELOMERASE CATALYTIC SUBUNIT

[75] Inventors: Thomas R. Cech; Joachim Lingner, both of Boulder, Colo.

[73] Assignees: University Technology Corporation, Boulder, Colo.; Geron Corporation, Menlo Park, Calif.

[21] Appl. No.: 08/974,549

[22] Filed: Nov. 19, 1997

Related U.S. Application Data

[63] Continuation-in-part of application No. 08/915,503, Aug. 14, 1997, abandoned, and a continuation-in-part of application No. 08/912,951, Aug. 14, 1997, and a continuation-in-part of application No. 08/911,312, Aug. 14, 1997, which is a continuation-in-part of application No. 08/854,050, May 9, 1997, which is a continuation-in-part of application No. 08/851,843, May 6, 1997, which is a continuation-in-part of application No. 08/846,017, Apr. 25, 1997, which is a continuation-in-part of application No. 08/844,419, Apr. 18, 1997, which is a continuation-in-part of application No. 08/724,643, Oct. 1, 1996.

[30] Foreign Application Priority Data

Oct. 1, 1997 [WO] WIPO PCT/US97/17618
Oct. 1, 1997 [WO] WIPO PCT/US97/17885

[51] Int. Cl.[7] A61K 38/00; C07K 5/00; C07K 7/00; C07K 16/00

[52] U.S. Cl. 530/324; 530/827; 530/828; 536/23.2; 536/23.5

[58] Field of Search 530/324, 827, 530/828; 536/23.2, 23.5

[56] References Cited

FOREIGN PATENT DOCUMENTS

WO 98/45450 10/1998 WIPO C12N 15/54

Primary Examiner—Yvonne Eyler
Attorney, Agent, or Firm—Townsend and Townsend and Crew LLP

[57] ABSTRACT

The invention provides compositions and methods related to telomerase reverse transcriptase, the catalytic protein subunit of human telomerase. The polynucleotides and polypeptides of the invention are useful for diagnosis, prognosis and treatment of human diseases, for changing the proliferative capacity of cells and organisms, and for identification and screening of compounds and treatments useful for treatment of diseases such as cancers.

1 Claim, 103 Drawing Sheets

-continued

```
                885                 890                 895

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            900                 905                 910

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        915                 920                 925

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Xaa Xaa Gly Xaa
    930                 935                 940

Xaa Gln Gly Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
945                 950                 955                 960

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                965                 970                 975

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            980                 985                 990

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Asp Xaa Leu Xaa Xaa Xaa Xaa
        995                 1000                1005

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    1010                1015                1020

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys
1025                1030                1035                1040
```

What is claimed is:

1. An isolated polypeptide consisting of the amino acid sequence shown in SEQ. ID. NO. 110.

* * * * *

US006261836B1

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,261,836 B1**
(45) **Date of Patent: *Jul. 17, 2001**

(54) **TELOMERASE**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**; **Calvin B. Harley**, both of Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **University Technology Corporation**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/854,050**

(22) Filed: **May 9, 1997**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned.

(51) **Int. Cl.**[7] **C07H 21/04**; A61K 38/00; C07K 16/00; C07K 17/00

(52) **U.S. Cl.** 435/325; 435/320.1; 435/7.1; 435/7.2; 530/324; 530/350; 514/2; 536/23.2; 536/23.5

(58) **Field of Search** 435/6, 7.23, 325, 435/320.1, 7.1, 7.2; 530/324, 350; 514/2; 536/23.2, 23.5

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	6/1974	Tanenholtz et al.	195/103.5
3,850,752	11/1974	Schuurs et al.	195/103.5
3,939,350	2/1976	Kronick et al.	250/365
3,996,345	12/1976	Ullman et al.	424/12
4,275,149	6/1981	Litman et al.	435/7
4,277,437	7/1981	Maggio	422/61
4,366,241	12/1982	Tom et al.	435/7
4,683,195	7/1987	Mullis et al.	435/6
4,683,202	7/1987	Mullis	435/91
4,816,567	3/1989	Cabilly et al.	530/387
4,965,188	10/1990	Mullis et al.	435/6
5,489,508	2/1996	West et al.	435/6
5,583,016	12/1996	Villeponteau et al.	435/91.3
5,747,317	5/1998	Cao .	
5,770,422	6/1998	Collins .	
6,093,809	7/2000	Cech et al. .	

FOREIGN PATENT DOCUMENTS

9154575	6/1997	(JP) .	
WO 93/23572	11/1993	(WO) .	
WO 95/13382	5/1995	(WO) .	
WO 96/01835	1/1996	(WO) .	
WO 96/12811	5/1996	(WO) .	
WO 96/19580	6/1996	(WO) .	
WO 96/40868	12/1996	(WO) .	
WO 98/01542	1/1998	(WO) .	
WO 98/01543	1/1998	(WO) .	
WO 98/08938	2/1998	(WO) .	
WO 98/07838	3/1998	(WO) .	
WO 98/21343	5/1998	(WO) .	
WO 98/23759	6/1998	(WO) .	
WO 98/37181	8/1998	(WO) .	
WO 98/45450	10/1998	(WO)	C12N/15/54
WO98/59040	12/1998	(WO)	C12N/9/00
WO99/01560	1/1999	(WO)	C12N/15/54

OTHER PUBLICATIONS

Hillier, et al., Direct Submission to GenBank, EST Database, Accession No. W70315, Available Oct. 17, 1996.*

Feng et al., "The RNA Componant of Human Telomerase," 1995, *Science* 269:1236.

Zakian, "Telomeres: Beginning to Understand the End," *Science* 270:1601 [1995].

Blackburn and Gall, "A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena," *J. Mol. Biol.*, 120:33 [1978].

Oka et al., "Inverted terminal repeat sequence in the macronuclear DNA of *Stylonychia pustulata*," *Gene* 10:301 [1980].

Klobutcher et al., "All gene-sized DNA molecules in four species of hypotrichs have the same terminal sequence and an unusual 3' terminus," *Proc. Natl. Acad. Sci.*, 78:3015 [1981].

Lingner et al., "Telomerase RNAs of different ciliates have a common secondary structure and a permuted template," *Genes Develop.*, 8:1984 [1994].

Biessmann et al., "Addition of Telomere-Associated HeT DNA Sequences "Heals" Broken Chromosome Ends in Drosophila," *Cell* 61:663 [1990].

(List continued on next page.)

Primary Examiner—Yvonne Eyler
Assistant Examiner—Janet Andies
(74) *Attorney, Agent, or Firm*—David J. Earp; Randolph T. Apple; William M. Smith

(57) **ABSTRACT**

The present invention is directed to telomerase nucleic acids and amino acids. In particular, the present invention is directed to nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs, including the 123 kDa and 43 kDa telomerase protein subunits of *Euplotes aediculatus*, and related sequences from Schizosaccharomyces, Saccharomyces sequences, and human telomerase. The present invention is also directed to polypeptides comprising these telomerase protein subunits, as well as functional polypeptides and ribonucleoproteins that contain these subunits.

12 Claims, 81 Drawing Sheets

-continued

```
Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu
            900                 905                 910

Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe
        915                 920                 925

Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
    930                 935                 940

Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945                 950                 955                 960

Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
                965                 970                 975

Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
            980                 985                 990

Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
        995                 1000                1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
    1010                1015                1020

Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

We claim:

1. A synthetic or recombinant human telomerase reverse transcriptase (hTRT) protein, or a variant thereof, or a fragment thereof, wherein said variant is encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide having a sequence complementary to SEO ID NO: 224, and wherein said hTRT protein, variant, or fragment has telmerase catalytic activity when complexed with a telomerase RNA.

2. A composition comprising the hTRT protein of claim 1, and further comprising an RNA, wherein the hTRT protein and the RNA form a telomerase ribonucleic acid complex.

3. An isolated, synthetic, substantially pure, or recombinant polynucleotide comprising a nucleic acid sequence that encodes the hTRT protein, variant or fragment of claim 1, or the complement of said nucleic acid sequence.

4. The polynucleotide of claim 1, comprising a promoter sequence operably linked to the sequence encoding the hTRT protein.

5. A isolated cell comprising the recombinant polynucleotide of claim 3.

6. A cell of claim 5 that is a eukaryotic cell.

7. An isolated, synthetic, substantially pure, or recombinant polynucleotide encoding a full-length naturally occurring human telomerase reverse transcriptase (hTRT) protein, said protein having 1132 amino acid residues.

8. An isolated, synthetic, substantially pure, or recombinant polynucleotide encoding a full-length naturally occurring human telomerase reverse transcriptase (hTRT) protein, said protein having 1132 amino acid residues, wherein said polynucleotide comprises the hTRT protein encoding sequence of bases 56 to 3451 of Seq. ID. No. 224 (FIG. 53).

9. The polynucleotide of claim 3, wherein the encoded protein has 1132 amino acid residues.

10. The polynucleotide of claim 9, wherein said polynucleotide comprises an encoding region of bases 56–3451 of SEQ ID NO: 224.

11. A method of preparing recombinant telomerase, said method comprising contacting the recombinant hTRT protein of claim 1 with a telomerase RNA component under conditions such that said recombinant protein and said telomerase RNA component associate to form a telomerase enzyme capable of catalyzing the addition of nucleotides to a telomerase substrate.

12. The method of claim 11, wherein said contacting occurs in a cell which has been engineered to express recombinant hTRT.

* * * * *

AX

US006309867B1

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,309,867 B1**
(45) **Date of Patent: Oct. 30, 2001**

(54) TELOMERASE

(75) Inventors: **Thomas R. Cech**; **Toru Nakamura**, both of Boulder, CO (US)

(73) Assignee: **University Technology Corporation**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/430,323**

(22) Filed: **Oct. 29, 1999**

Related U.S. Application Data

(63) Continuation of application No. 08/854,050, filed on May 9, 1997, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned.

(51) **Int. Cl.**[7] **C12N 9/12**
(52) **U.S. Cl.** **435/194**; 435/194
(58) **Field of Search** 435/194

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,093,809 * 7/2000 Cech et al. 536/23.5

OTHER PUBLICATIONS

Nakamura et al, Telomerase catalytic subunit homologs from fission yeast and human, Science, 1997, 277, 955–959.*

* cited by examiner

Primary Examiner—Nashaat T. Nashed
Assistant Examiner—Malgorzata A. Walicka
(74) *Attorney, Agent, or Firm*—David J. Earp; Randolph T. Apple; William M. Smith

(57) **ABSTRACT**

The present invention is directed to novel telomerase nucleic acids and amino acids. In particular, the present invention is directed to nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs, including the 123 kDa and 43 kDa telomerase protein subunits of *Euplotes aediculatus*, and related sequences from Schizosaccharomyces, Saccharomyces sequences, and human telomerase. The present invention is also directed to polypeptides comprising these telomerase protein subunits, as well as functional polypeptides and ribonucleoproteins that contain these subunits.

1 Claim, 78 Drawing Sheets

-continued

```
Pro Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu
785                 790                 795                 800

Ala Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His
                805                 810                 815

Ala Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro
            820                 825                 830

Gln Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp
        835                 840                 845

Met Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu
    850                 855                 860

Arg Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala
865                 870                 875                 880

Lys Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys
                885                 890                 895

Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu
            900                 905                 910

Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe
        915                 920                 925

Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
    930                 935                 940

Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945                 950                 955                 960

Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
                965                 970                 975

Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
            980                 985                 990

Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
        995                 1000                1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
    1010                1015                1020

Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

We claim:

1. An isolated polypeptide consisting of the amino acid sequence shown in SEQ. ID. NO. 69.

* * * * *

US006444650B1

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,444,650 B1**
(45) **Date of Patent: *Sep. 3, 2002**

(54) **ANTISENSE COMPOSITIONS FOR DETECTING AND INHIBITING TELOMERASE REVERSE TRANSCRIPTASE**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**; **Calvin B. Harley**, both of Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **University Technology Corporation**, Boulder, CO (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/052,919**

(22) Filed: **Mar. 31, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/974,549, filed on Nov. 19, 1997, now Pat. No. 6,166,178, and a continuation-in-part of application No. 08/974,584, filed on Nov. 19, 1997, which is a continuation-in-part of application No. 08/915,503, filed on Aug. 14, 1997, now abandoned, which is a continuation-in-part of application No. 08/912,951, filed on Aug. 14, 1997, which is a continuation-in-part of application No. 08/911,312, filed on Aug. 14, 1997, now abandoned, which is a continuation-in-part of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned, application No. 09/052,919, which is a continuation-in-part of application No. PCT/US97/17885, filed on Oct. 1, 1997, and a continuation-in-part of application No. PCT/US97/17618, filed on Oct. 1, 1997.

(51) **Int. Cl.**[7] **A01N 43/04**; A61K 31/70; C02H 21/04

(52) **U.S. Cl.** **514/44**; 536/23.2; 536/23.5

(58) **Field of Search** 536/23.2, 23.5; 514/44

(56) References Cited

U.S. PATENT DOCUMENTS

5,583,016 A 12/1996 Villeponteau

5,639,613 A 6/1997 Shay

FOREIGN PATENT DOCUMENTS

WO WO97/38013 10/1997

OTHER PUBLICATIONS

Gura, Science vol. 270, pp 575–577, 1995.*

EST, Accession No. AA281296, NCBI database, 1997.

EST, Accession No. AA311750, NCBI database, 1997.

EST, Accession No. AA299878, NCBI database, 1997.

Harrington, Lea, et al., (1997) "A Mammalian Telomerase–Associated Protein", *Science* 275:973–977.

Langford, Lauren A., et al. (1997) "Telomerase Activity in Ordinary Meningiomas Predicts Poor Outcome", *Human Pathology* 28(4):416–420.

Nakamura, Toru, M., et al. (1997) "Telomerase Catalytic Subunit Homologs from Fission Yeast and Human", *Science.* 277:955–959.

Harrington, Lea, et al. (1995) "Gel Shift and UV Cross–linking Analysis of *Tetrahymena* Telomerase", *The Journal of Biological Chemistry,* 270(15):8893–8901.

Collins, Kathleen, et al. (1995) "Purification of Tetrahymena Telomerase and Cloning of Genes Encoding of Two Protein Components of the Enzyme", *Cell,* 81:677–686.

Greider, Carol W. (1998) Telomerase and senescence: The history, experiment, the future, *Current Biology,* 8(5):R178–R181.

Kilian, Andrzej, et al. (1997) "Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types", *Human Molecular Genetics,* 6(12):2011–2019.

Meyerson, Matthew, et al. (1970) "hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up–Regulated in Tumor Cells and during Immortalization", *Cell* 90:785–795.

Morin, G.B. (1997) "The Implications of Telomerase Biochemistry for Human Disease", *European Journal of Cancer,* 33(5):750–760.

* cited by examiner

Primary Examiner—Yvonne Eyler
Assistant Examiner—Janet L. Andres
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Townsend and Townsend and Crew LLP

(57) **ABSTRACT**

The present invention provides TRT antisense oligonucleotides, methods of detecting TRT, methods of diagnosing telomerase-related conditions, methods of diagnosing and providing a prognosis for cancer, and methods of treating telomerase-related conditions, including cancer.

14 Claims, 3 Drawing Sheets

-continued

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "phosphorothioate oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GCGGGTGGCC ATCAGTCCAG GATGGTCTTG 30

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "phosphorothioate oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CAGACTCCCA GCGGTGCGGG CCTGGGTGTG 30

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "phosphorothioate oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

AGCCGGACAC TCAGCCTTCA GCCGGACATG 30

What is claimed is:

1. An isolated antisense oligonucleotide that hybridizes to a target DNA having the nucleotide sequence of SEQ. ID NO:1 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl;

wherein T_m is the melting temperature of a complementary oligonucleotide hybridized to the target DNA in aqueous solution at 1 M NaCl, wherein the complementary oligonucleotide is exactly complementary to SEQ. ID NO:1 and the same length as the antisense oligonucleotide; and

wherein hybridization of the antisense oligonucleotide to an mRNA encoding hTRT (SEQ. ID NO:1.)inhibits expression of the mRNA.

2. The oligonucleotide of claim 1 that hybridizes to the target DNA at 5° C. below T_m.

3. The oligonucleotide of claim 1 that is DNA.

4. The oligonucleotide of claim 1 that is RNA.

5. The oligonucleotide of claim 1 that comprises one or more synthetic nucleotides.

6. The oligonucleotide of claim 5 that comprises a phosphorothioate oligonucleotide.

7. The oligonucleotide of claim 1 that is from 20 to 100 nucleotides in length.

8. The oligonucleotide of claim 7 that is 30 nucleotides in length.

9. The oligonucleotide of claim 1 that is from 10 to 50 nucleotides in length.

10. The oligonucleotide of claim 1 that comprises a sequence of about 7 to about 100 nucleotides that is exactly complementary to SEQ. ID NO:1.

11. The oligonucleotide of claim 10 that is from 20 to 100 nucleotides in length.

12. The oligonucleotide of claim 11, wherein the oligonucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:4–72.

13. The oligonucleotide of claim 12, that is 30 nucleotides in length.

14. The oligonucleotide of claim 1, wherein said oligonucleotide reduces telomerase activity in a cell by at least 50%.

* * * * *

US006475789B1

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,475,789 B1**
(45) **Date of Patent: *Nov. 5, 2002**

(54) **HUMAN TELOMERASE CATALYTIC SUBUNIT: DIAGNOSTIC AND THERAPEUTIC METHODS**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**, Palo Alto, CA (US); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **University Technology Corporation**, Boulder, CO (US); **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/912,951**

(22) Filed: **Aug. 14, 1997**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/845,050, filed on May 9, 1997, now Pat. No. 5,743,518, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned.

(51) **Int. Cl.**[7] C12N 5/08; C12N 15/12; C07H 21/04; A61K 38/43

(52) **U.S. Cl.** **435/366**; 435/320.1; 435/69.1; 536/23.2; 424/94.1

(58) **Field of Search** 435/366, 320, 435/69.1; 536/23.2; 429/94.1

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tanenholtz et al.	195/103.5
3,850,752	A	11/1974	Schuurs et al.	195/103.5
3,939,350	A	2/1976	Kronick et al.	250/365
3,996,345	A	12/1976	Ullman et al.	424/12
4,275,149	A	6/1981	Litman et al.	435/7
4,277,437	A	7/1981	Maggio	422/61
4,366,241	A	12/1982	Tom et al.	435/7
4,683,195	A	7/1987	Mullis et al.	435/6
4,683,202	A	7/1987	Mullis	435/91
4,816,567	A	3/1989	Cabilly et al.	530/387
4,965,188	A	10/1990	Mullis et al.	435/6
5,489,508	A	2/1996	West et al.	435/6
5,583,016	A	12/1996	Villeponteau et al.	435/91.3
5,747,317	A	5/1998	Cao	
5,770,422	A	6/1998	Collins	
6,258,535	B1	7/2001	Villeponteau et al.	
6,261,556	B1	7/2001	Weinrich et al.	
6,261,836	B1	7/2001	Cech et al.	

FOREIGN PATENT DOCUMENTS

JP	09154575 A	6/1997
WO	WO 84/03564	9/1984
WO	WO 95/13382	5/1995
WO	WO 96/01835	1/1996
WO	WO 96/12811	5/1996
WO	WO 96/19580	6/1996
WO	WO 96/40868	12/1996
WO	WO 98/01542	1/1998
WO	WO 98/01543	1/1998
WO	WO 98/08938	2/1998
WO	WO 98/07838	3/1998
WO	WO 98/21343	5/1998
WO	WO 98/37181	8/1998
WO	WO 98/45450	10/1998

OTHER PUBLICATIONS

Johnson et al., Mol. Cell. Biol. 1991, vol. 11, pp. 1–11.*

Counter et al., Proc. Nat. Acad. Sci. 1994, vol. 91, pp. 2900–2904.*

Zakian, "Telomeres: Beginning to Understand the End," *Science* 270:1601 [1995].

Blackburn and Gall, "A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena," *J. Mol. Biol.*, 120:33 [1978].

Oka et al.,"Inverted terminal repeat sequence in the macronuclear DNA of *Stylonychia pustulata*," *Gene* 10:301 [1980].

Klobutcher et al.,"All gene-sized DNA molecules in four species of hypotrichs have the same terminal sequence and an unusual 3' terminus," *Proc. Natl. Acad. Sci.*, 78:3015 [1981].

Lingner et al.,"Telomerase RNAs of different ciliates have a common secondary structure and a permuted template," *Genes Develop.*, 8:1984 [1994].

Biessmann et al., "Addition of Telomere-Associated HeT DNA Sequences "Heals" Broken Chromosome Ends in Drosophilia," *Cell* 61:663 [1990].

Sheen and Levis, "Transposition of the LINE-like retrotransposon TART to Drosophila chromosome termini," *Proc. Natl. Acad. Sci.*, 91:12510 [1994].

Kipling and Cooke, "Hypervariable ultra-long telomeres in mice," *Nature* 347:400 [1990].

(List continued on next page.)

Primary Examiner—Yvonne Eyler
Assistant Examiner—Janet L. Andres
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Scott L. Ausenhus

(57) **ABSTRACT**

The invention provides compositions and methods related to human telomerase reverse transcriptase (hTRT), the catalytic protein subunit of human telomerase. The polynucleotides and polypeptides of the invention are useful for diagnosis, prognosis, and treatment of human diseases, for changing the proliferative capacity of cells and organisms, and for identification and screening of compounds and treatments useful for treatment of diseases such as cancers.

8 Claims, 34 Drawing Sheets

-continued

```
Gln Val Asn Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu
1265                1270                1275                1280

Leu Leu Gln Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe
                1285                1290                1295

His Gln Gln Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser
            1300                1305                1310

Asp Thr Ala Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly
        1315                1320                1325

Met Ser Leu Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala
    1330                1335                1340

Val Gln Trp Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His
1345                1350                1355                1360

Arg Val Thr Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr
                1365                1370                1375

Gln Leu Ser Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala
            1380                1385                1390

Ala Ala Asn Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
        1395                1400                1405
```

(2) INFORMATION FOR SEQ ID NO:335:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:335:

```
Gly Ser Thr His Ile Ser His Ile Ser His Ile Ser His Ile Ser His
  1               5                  10                  15

Ile Ser His Ile Ser His Ile Ser His Ile Ser
            20                  25
```

What is claimed is:

1. A mammalian cell that contains a recombinant polynucleotide comprising a nucleic acid sequence that encodes a telomerase reverse transcriptase protein, variant, or fragment having telomerase catalytic activity when complexed with a telomerase RNA, wherein said recombinant polynucleotide hybridizes to a DNA having a sequence complementary to SEQ ID NO: 1 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl,

wherein T_m is the melting temperature of a complementary polynucleotide hybridized to said DNA in aqueous solution at 1M NaCl, wherein the complementary polynucleotide is exactly complementary to SEQ ID NO: 1 and is the same length as the recombinant polynucleotide.

2. The mammalian cell of claim 1, wherein the recombinant polynucleotide encodes a full-length naturally occurring human telomerase reverse transcriptase.

3. The mammalian cell of claim 2, which expresses said encoding sequence at the mRNA level, as measured by PCR amplification.

4. The mammalian cell of claim 1, which expresses said encoding sequence at the protein level, as measured by immunoassay.

5. The mammalian cell of claim 1, which has telomerase activity, as measured in a primer elongation assay.

6. The mammalian cell of claim 1, which is a human cell.

7. The mammalian cell of claim 6, which is a stem cell.

8. The mammalian cell of claim 1, which is a stem cell.

* * * * *

US006617110B1

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,617,110 B1**
(45) **Date of Patent: Sep. 9, 2003**

(54) **CELLS IMMORTALIZED WITH TELOMERASE REVERSE TRANSCRIPTASE FOR USE IN DRUG SCREENING**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**, Palo Alto, CA (US); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Richmood, CA (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **University Technology Corporation**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/721,456**

(22) Filed: **Nov. 24, 2000**

Related U.S. Application Data

(63) Continuation of application No. 08/974,549, filed on Nov. 19, 1997, now Pat. No. 6,166,178, which is a continuation-in-part of application No. 08/915,503, filed on Aug. 14, 1997, now abandoned, which is a continuation-in-part of application No. 08/912,951, filed on Aug. 14, 1997, now Pat. No. 6,475,789, and a continuation-in-part of application No. 08/911,312, filed on Aug. 14, 1997, now abandoned, which is a continuation-in-part of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned.

(51) **Int. Cl.**[7] **C12G 1/68**; C12N 9/12; C12N 15/09; C12N 5/00; C12Q 1/02

(52) **U.S. Cl.** **435/6**; 435/194; 435/69.2; 435/325; 435/29; 536/23.2

(58) **Field of Search** 435/194, 6, 325, 435/69.2, 29; 536/23.1, 23.2, 23.5

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tanenholtz et al.
3,850,752	A	11/1974	Schuurs et al.
3,939,350	A	2/1976	Kronick et al.
3,996,345	A	12/1976	Ullman et al.
4,275,149	A	6/1981	Litman et al.
4,277,437	A	7/1981	Maggio
4,366,241	A	12/1982	Tom et al.
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,816,567	A	3/1989	Cabilly et al.
4,965,188	A	10/1990	Mullis et al.
5,489,508	A	2/1996	West et al.
5,583,016	A	12/1996	Villeponteau et al.
5,747,317	A	5/1998	Cao
5,770,422	A	6/1998	Collins
6,093,809	A	7/2000	Cech et al.
6,258,535	B1	7/2001	Villeponteau et al.
6,261,556	B1	7/2001	Weinrich et al.
6,261,836	B1	7/2001	Cech et al.

FOREIGN PATENT DOCUMENTS

JP	09154575 A	6/1987
WO	WO 98/08938	2/1908
WO	WO 84/03584	9/1984
WO	WO 95/13382	5/1995
WO	WO 98/01835	1/1996
WO	WO 98/12811	5/1996
WO	WO 98/19580	6/1996
WO	WO 96/40868	12/1996
WO	WO 98/01542	1/1998
WO	WO 98/01543	1/1998
WO	WO 98/07838	3/1998
WO	WO 98/21343	5/1998
WO	WO 98/37181	8/1998
WO	WO 98/45450	10/1998
WO	WO98/59040	12/1998
WO	WO99/01560	1/1999

OTHER PUBLICATIONS

U.S. patent application Ser. No. 08/751,189, Harrington et al., filed Nov. 15, 1996.

U.S. patent application Ser. No. 60/038,760, Counter et al., filed Feb. 20, 1997.

1994 Genome Issue of Science (265:1981f).

Anderson and Young, "Quantitative Filter Hybridization" in *Nucleic Acid Hybridization* pp. 73–111 (1985).

Ausubel et al., Current Protocols In Molecular Biology, John Wiley & Sons, New York NY (1989).

Autexier et al., "Reconstitution of human telomerase activity and Identification of a minimal functional region of the human telomease RNA," (1998) EMBO J, 15:5928.

Auxexier and Grelder, "Functional reconstitution of wild-type and mutant Tetrahymena telomerase," (1994) *Genes Develop.*, 8:583.

Berger and Kimmel, *Guide to Molecualar Cloning Techniques*, Meth. Enzymol., vol. 152, Academic Press, San Diego CA (1987).

Biessman et al., "Addition of Telomere–Associated HeT DNA Sequences "Heals" Broken Chromosome End is in Drosophila," (1990) *Cell*, 61:683.

(List continued on next page.)

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—M. Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Scott L. Ausenhus

(57) **ABSTRACT**

The invention provides compositions and methods related to human telomerase reverse transcriptase (hTRT), the catalytic protein subunit of human telomerase. The polynucleotides and polypeptides of the invention are useful for diagnosis, prognosis and treatment of human diseases, for changing the proliferative capacity of cells and organisms, and for identification and screening of compounds and treatments useful for treatment of diseases such as cancers.

39 Claims, 103 Drawing Sheets

SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/sequence.html?DocID=6617110B1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A method of drug screening or validation of a drug, comprising:

a) obtaining a drug or drug candidate,

b) obtaining a cultured mammalian cell comprising a nucleic acid sequence that encodes a telomerase reverse transcriptase protein, variant, or fragment, wherein said variant, or fragment, has telomerase catalytic activity when complexed with a telomerase RNA, wherein the nucleic acid sequence hybridizes under stringent conditions to a polynucleotide having a sequence complementary to SEQ ID NO: 1, and wherein the expression of the protein, variant, or fragment in the cell increases the number of divisions the cell can undergo before senescence;

c) administering the drug or drug candidate to the cultured cell; and

d) determining if the drug or candidate has an effect on the cell.

2. The method of claim 1, wherein the cell is a human cell.

3. The method cell of claim 2, wherein the cell further comprises a selectable marker gene.

4. The method of claim 2, wherein the nucleic acid comprises a constitutive promoter.

5. The method of claim 2, wherein the nucleic acid comprises an inducible promoter.

6. The method of claim 2, wherein the cell is a liver cell.

7. The method of claim 6, wherein the cell is a hepatocyte.

8. The method of claim 2, wherein the cell is a nerve cell.

9. The method of claim 8, wherein the cell is a glial cell, astrocyte, or oligodendrocyte.

10. The method of claim 8, wherein the cell is a neuron of the central nervous system.

11. The method of claim 10, wherein the cell is a cholinergic or adrenergic cell.

12. The method of claim 2, wherein the cell is a retinal pigmented epithelial cell.

13. The method of claim 2, wherein the cell is a contractile cell.

14. The method of claim 13, wherein the cell is a heart muscle cell or smooth muscle cell.

15. The method of claim 2, wherein the cell is a fat cell.

16. The method of claim 2, wherein the cell is a fibroblast.

17. The method of claim 2, wherein the cell is a vascular endothelial cell.

18. The method of claim 2, wherein the cell is a hormone secreting cell.

19. The method of claim 18, wherein the cell secretes insulin or glucagon.

20. The method of claim 18, wherein the cell is a pituitary cell, thyroid hormone secreting cell, or adrenal cell.

21. The method of claim 2, wherein the cell is a fat storing cell.

22. The method of claim 2, wherein the cell is an epithelial or mucosal cell.

23. The method of claim 22, wherein the cell is an oral cavity cell, stomach cell, or intestinal cell.

24. The method of claim 22, wherein the cell is a mammary gland, uterus, or prostate cell.

25. The method of claim 22, wherein the cell is an air space epithelial cell or the lung.

26. The method of claim 2, wherein the cell is a tubular cell of the kidney.

27. The method of claim 2, wherein the cell is a blood cell or a cell of the immune system,

28. The method of claim 27, wherein the cell is a T or B lymphocyte.

29. The method of claim 27, wherein the cell is a mast cell or eosinophil.

30. The method of claim 27, wherein the cell is a monocyte or macrophage.

31. The method of claim 2, wherein the cell is an osteoblast, osteocyte, or osteoclast.

32. The method of claim 2, wherein the cell is a chondrocyte or synovial cell.

33. The method of claim 2, wherein the cell is a stem cell.

34. The method of claim 33, wherein the cell is an embryonic stem cell.

35. The method of claim 33, wherein the cell is an embryonic germ cell.

36. The method of claim 33, wherein the cell is an adult stem cell.

37. The method of claim 2, wherein the nucleic acid encodes a full-length, naturally occurring human telomerase reverse transcriptase.

38. The method of claim 2, wherein the nucleic acid encodes a full-length, naturally occurring human telomerase reverse transcriptase having the amino acid sequence of SEQ ID NO: 2.

39. The method of claim 1, comprising determining whether the drug or drug candidate is lethal to the cell.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

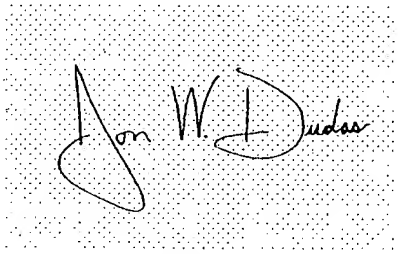
PATENT NO. : 6,617,110 B1
DATED : September 9, 2003
INVENTOR(S) : Thomas R. Cech

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [*] Notice, "0 days" should read -- 306 days --.
Item [22], PCT Filed, "**Nov. 24, 2000**" should read -- **Nov. 22, 2000** --.
Item [63], **Related U.S. Application Data**, the phrase "which is" should read -- all three of which are --

Signed and Sealed this

Seventh Day of June, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

US006627619B2

(12) **United States Patent**
Cech et al.

(10) Patent No.: **US 6,627,619 B2**
(45) Date of Patent: **Sep. 30, 2003**

(54) **ANTISENSE COMPOSITIONS FOR DETECTING AND INHIBITING TELOMERASE REVERSE TRANSCRIPTASE**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**, Palo Alto, CA (US); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **University Technology Corporation**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/953,052**

(22) Filed: **Sep. 14, 2001**

(65) **Prior Publication Data**

US 2002/0173476 A1 Nov. 21, 2002

Related U.S. Application Data

(62) Division of application No. 09/052,919, filed on Mar. 31, 1998, now Pat. No. 6,444,650, which is a continuation-in-part of application No. 08/974,549, filed on Nov. 19, 1997, now Pat. No. 6,166,178, and a continuation-in-part of application No. 08/974,584, filed on Nov. 19, 1997, which is a continuation-in-part of application No. 08/915,503, filed on Aug. 14, 1997, now abandoned, and a continuation-in-part of application No. 08/912,951, filed on Aug. 14, 1997, now Pat. No. 6,475,789, and a continuation-in-part of application No. 08/911,312, filed on Aug. 14, 1997, now abandoned, which is a continuation-in-part of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned, and a continuation-in-part of application No. PCT/US97/17885, filed on Oct. 1, 1997, and application No. PCT/US97/17618, filed on Oct. 1, 1997.

(51) **Int. Cl.**[7] **A01N 43/04**; C12N 9/10; C12N 9/12; C07H 21/04; C12Q 1/68

(52) **U.S. Cl.** **514/44**; 435/193; 435/194; 435/6; 536/23.2; 536/24.5; 536/23.5

(58) **Field of Search** 435/6, 193, 194; 536/23.2, 23.5, 24.5; 514/44

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,583,016	A	12/1996	Villeponteau et al.	
5,639,613	A	6/1997	Shay	
6,093,809	A *	7/2000	Cech et al.	536/23.5
6,166,178	A *	12/2000	Cech et al.	530/342
6,309,867	B1 *	10/2001	Cech et al.	435/194
6,444,650	B1 *	9/2002	Cech et al.	514/44

FOREIGN PATENT DOCUMENTS

WO	WO 97/38013	10/1997

OTHER PUBLICATIONS

Adams M. et al. Initial Assessment of Human Gene Diversity and Expression Patterns Based upon 83 Million Nucleotides of cDNA Sequence. Nature, 377, supp. Sep. 28, 1995, 3–174.*

Lanfranchi G. et al. Identification of 4370 Expressed Sequence Tags from a 3'–End–Specific cDNA library of Human Skeletal Muscle by DNA Sequencing and Filter Hybridization, Genome Res., 6, 1996, 35–42.*

Bonaldo M. et al. Normalization and Subtraction: Two Approaches to Facilitate Gene Discovery, Genome Res. 6, 1996, 791–806.*

Harrington, Lea et al. (1997) "A Mammalian Telomerase–Associated Protein", *Science* 275:973–977.

Langford, Lauren A., et al. (1997) "Telomerase Activity in Ordinary Meningiomas Predicts Poor Outcome", *Human Pathology* 28(4):416–420.

Nakamura, Toru, M., et al. (1997) "Telomerase Catalytic Subunit Homologs from Fission Yeast and Human", *Science.* 277:955–959.

Harrington, Lea, et al. (1995) "Gel Shift and UV Cross–linking Analysis of *Tetrahymena* Telomerase", *The Journal of Biological Chemistry*, 270(15):8893–8901.

Collins, Kathleen, et al. (1995) "Purification of Tetrahymena Telomerase and Cloning of Genes Encoding the Two Protein Components of the Enzyme", *Cell*, 81:677–686.

Greider, Carol W. (1998) Telomerase and senescence: The history, experiment, the future, *Current Biology*, 8(5):R178–R181.

Kilian, Andrzej, et al. (1997) "Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types", *Human Molecular Genetics*, 6(12):2011–2019.

Meyerson, Matthew, et al. (1997) "*hEST2*, the Putative Human Telomerase Catalytic Subunit Gene, Is Up–Regulated in Tumor Cells and during Immortalization", *Cell* 90:785–795.

Morin, G.B. (1997) "The Implications of Telomerase Biochemistry for Human Disease", *European Journal of Cancer*, 33(5):750–760.

EST, Accession No. AA281296, NCBI database, 1997.

EST, Accession No. AA311750, NCBI database, 1997.

EST, Accession No. AA299878, NCBI database, 1997.

* cited by examiner

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—Malgorzata A. Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Townsend & Townsend & Crew LLP

(57) **ABSTRACT**

The present invention provides TRT antisense oligonucleotides, methods of detecting TRT, methods of diagnosing telomerase-related conditions, methods of diagnosing and providing a prognosis for cancer, and methods of treating telomerase-related conditions, including cancer.

26 Claims, 3 Drawing Sheets

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

GCGTTCTTGG CTTTCAGGAT GGAGTAGCAG 30

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "phosphorothioate oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

GCGGGTGGCC ATCAGTCCAG GATGGTCTTG 30

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "phosphorothioate oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

CAGACTCCCA GCGGTGCGGG CCTGGGTGTG 30

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "phosphorothioate oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

AGCCGGACAC TCAGCCTTCA GCCGGACATG 30

What is claimed is:

1. A method for inhibiting expression of human telomerase reverse transcriptase (hTRT) protein in a cell, comprising contacting the cell with an antisense oligonucleotide that hybridizes to a target DNA having the nucleotide sequence of SEQ ID NO:1 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl;

wherein T_m is the melting temperature of a complementary oligonucleotide hybridized to the target DNA in aqueous solution at 1 M NaCl, wherein the complementary oligonucleotide is exactly complementary to SEQ ID NO:1 and the same length as the antisense oligonucleotide; and

wherein hybridization of the antisense oligonucleotide to an mRNA encoding hTRT (SEQ ID NO:1) inhibits expression of the mRNA.

2. The method of claim 1, wherein the antisense oligonucleotide hybridizes to the target DNA at 5° C. below T_m.

3. The method of claim 1, wherein the antis ease oligonucleotide is from 10 to 50 nucleotides in length.

4. The method of claim 1, wherein the antisense oligonucleotide is from 20 to 100 nucleotides in length.

5. The method of clam 1, wherein the antisense oligonucleotide comprises at least 20 nucleotides exactly complementary to SEQ ID NO:1.

6. The method of claim 1, wherein the antisense oligonucleotide comprises at least 30 nucleotides exactly complementary to SEQ ID NO:1.

7. The method of claim 1, wherein the antisense oligonucleotide is DNA.

8. The method of claim 1, wherein the antisense oligonucleotide is RNA.

9. The method of claim 1, wherein the antisense oligonucleotide contains one or more synthetic nucleotides.

10. The method of claim 1, wherein the antisense oligonucleotide contains one or more phosphorothioate oligonucleotides.

11. The method of claim 1, wherein the antisense oligonucleotide contains one or more phosphoramidate oligonucleotides.

12. The method of claim 1, wherein the antisense oligonucleotide is a ribozyme.

13. The method of claim 1, wherein the antisense oligonucleotide contains a sequence selected from SEQ ID NOs:4–72.

14. The method of claim 1, whereby expression of hTRT protein in the cell is reduced by at least 50%.

15. The method of claim 1, wherein the antisense nucleotide hybridizes to a target DNA consisting of the first 945 bp of SEQ. ID NO:1; and whereby expression of hTRT protein in the cell is reduced by at least 75%.

16. The method of claim 1, wherein the antisense nucleotide hybridizes to a target DNA consisting of the first 945 bp of SEQ. ID NO:1; and whereby expression of hTRT protein in the cell is reduced by at least 90%.

17. A method for inhibiting expression of human telomerase reverse transcriptase protein in a cell, comprising contacting the cell with a nucleic acid that contains at least 10 consecutive nucleotides exactly complementary to SEQ ID NO:1;

wherein hybridization of the nucleic acid to an mRNA encoding hTRT (SEQ ID NO:1) inhibits expression of the mRNA.

18. The method of claim 17, wherein the nucleic acid is from 20 to 100 nucleotides in length.

19. The method of claim 17, wherein the nucleic acid contains one or more synthetic nucleotides.

20. The method of claim 17, whereby expression of hTRT protein is reduced by at least 50%.

21. The method of claim 17, wherein the 10 consecutive nucleotides are exactly complementary to a sequence contained within the first 945 bp of SEQ. ID NO:1; and whereby expression of hTRT protein is reduced by at least 90%.

22. A method for inhibiting expression of human telomerase reverse transcriptase protein in a cell, comprising contacting the cell with a nucleic acid that contains at least 20 consecutive nucleotides exactly complementary to SEQ. ID NO:1;

wherein hybridization of the nucleic acid to an mRNA encoding hTRT (SEQ. ID NO:1) inhibits expression of the mRNA.

23. The method of claim 22, wherein the nucleic acid is from 20 to 100 nucleotides in length.

24. The method of claim 22, wherein the nucleic acid contains one or more synthetic nucleotides.

25. The method of claim 22, whereby expression of hTRT protein is reduced by at least 50%.

26. The method of claim 22, wherein the 20 consecutive nucleotides are exactly complementary to a sequence contained within the first 945 bp of SEQ. ID NO:1; and whereby expression of hTRT protein is reduced by at least 90%.

* * * * *

US006808880B2

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,808,880 B2**
(45) **Date of Patent: Oct. 26, 2004**

(54) **METHOD FOR DETECTING POLYNUCLEOTIDES ENCODING TELOMERASE**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**, Palo Alto, CA (US); **Calvin Harley**, Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **Regents of the University of Colorado**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 93 days.

(21) Appl. No.: **09/766,253**

(22) Filed: **Jan. 19, 2001**

(65) **Prior Publication Data**

US 2002/0187471 A1 Dec. 12, 2002

Related U.S. Application Data

(63) Continuation of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned.

(51) **Int. Cl.**[7] **C12Q 1/68**
(52) **U.S. Cl.** 435/6; 536/23.1; 536/23.5; 536/24.31; 536/24.32; 536/24.33
(58) **Field of Search** 536/24.32, 23.1, 536/23.5, 24.31, 24.33; 435/6, 91.2

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tanenholtz et al.
3,850,752	A	11/1974	Schuurs et al.
3,939,350	A	2/1976	Kronick et al.
3,998,345	A	12/1976	Ullman et al.
4,275,149	A	6/1981	Litman et al.
4,277,437	A	7/1981	Maggio
4,366,241	A	12/1982	Tom et al.
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,816,567	A	3/1989	Cabilly et al.
4,965,188	A	10/1990	Mullis et al.
5,489,508	A	2/1996	West et al.
5,583,016	A	12/1996	Villeponteau et al.
5,597,697	A	1/1997	Diamond
5,747,317	A	5/1998	Cao
5,770,422	A	6/1998	Collins
5,917,025	A	6/1999	Collins
5,919,656	A	7/1999	Harrington et al.
6,093,809	A	7/2000	Cech et al.
6,166,178	A	12/2000	Cech et al.
6,258,535	B1	7/2001	Villeponteau et al.
6,261,556	B1	7/2001	Weinrich et al.
6,261,836	B1	7/2001	Cech et al.
6,309,867	B1	10/2001	Cech et al.
6,337,200	B1	1/2002	Morin
6,440,735	B1	8/2002	Gaeta
6,444,650	B1	9/2002	Cech et al.
6,475,789	B1	11/2002	Cech et al.
6,517,834	B1	2/2003	Weinrich et al.
6,608,188	B1	8/2003	Tsuchiya et al.
6,610,839	B1	8/2003	Morin et al.
6,617,110	B1	9/2003	Cech et al.
6,627,619	B2	9/2003	Cech et al.
2002/0164786	A1	11/2002	Cech et al.
2003/0009019	A1	1/2003	Cech et al.
2003/0032075	A1	2/2003	Cech et al.
2003/0044953	A1	3/2003	Cech et al.
2003/0059787	A1	3/2003	Cech et al.
2003/0096344	A1	5/2003	Cech et al.
2003/0100093	A1	5/2003	Cech et al.

FOREIGN PATENT DOCUMENTS

GB	2317891	A	4/1998
JP	9154575	A2	6/1997
WO	WO 84/03564	A1	9/1984
WO	WO 93/23572	A1	11/1993
WO	WO 94/17210	A1	8/1994
WO	WO 95/13382	A1	5/1995
WO	WO 96/01835	A1	1/1996
WO	WO 96/12811	A1	5/1996
WO	WO 96/19580	A2	6/1996
WO	WO 96/40868	A1	12/1996
WO	WO 98/01542	A1	1/1998
WO	WO 98/01543	A1	1/1998
WO	WO 98/07838	A1	2/1998
WO	WO 98/08938	A1	3/1998
WO	WO 98/14592	A2	4/1998
WO	WO 98/14593	A2	4/1998
WO	WO 98/21343	A1	5/1998
WO	WO 98/23759	A2	6/1998

(List continued on next page.)

OTHER PUBLICATIONS

Schena et al. Proceedings of the National Academy of Sciences, USA (Oct. 1996) 93: 10614–10619.*

(List continued on next page.)

Primary Examiner—Carla J. Myers
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Scott L. Ausenhus

(57) **ABSTRACT**

The present invention is directed to novel telomerase nucleic acids and amino acids. In particular, the present invention is directed to nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs, including the 123 kDa and 43 kDa telomerase protein subunits of *Euplotes aediculatus*, and related sequences from Schizosaccharomyces, Saccharomyces sequences, and human telomerase. The present invention is also directed to polypeptides comprising these telomerase protein subunits, as well as functional polypeptides and ribonucleoproteins that contain these subunits.

8 Claims, 59 Drawing Sheets

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "motif B peptide from human telomerase core protein 1 (TCP1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:

Arg Ala Thr Ser Tyr Val Gln Cys Gln Gly Ile Pro Gln Gly Ser Ile
1 5 10 15

Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly
20 25

(2) INFORMATION FOR SEQ ID NO: 170:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: <Unknown>
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..22
(D) OTHER INFORMATION: /note= "motif C peptide from human telomerase core protein 1 (TCP1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:

Arg Arg Asp Gly Leu Leu Leu Arg Leu Val Asp Asp Phe Leu Leu Val
1 5 10 15

Thr Pro His Leu Thr His
20

(2) INFORMATION FOR SEQ ID NO: 171:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: <Unknown>
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /note= "motif D peptide from human telomerase core protein 1 (TCP1)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:

Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys Val Val
1 5 10 15

We claim:

1. A method for detecting the presence of polynucleotide sequences encoding at least a portion of telomerase in a biological sample, comprising the steps of:

a) obtaining an amino acid sequence encoded in a polynucleotide contained in a biological sample;

b) comparing the amino acid sequence with the telomerase amino acid motif W-X^{12}-FFY-X^{1}-TE, Wherein X is any amino acid; and then

c) determining that the sample contains a polynucleotide encoding at least a portion of telomerase if the sequence obtained in step a) contains said telomerase amino acid motif.

2. The method of claim 1 wherein the telomerase is a telomerase of a single-celled eukaryote.

3. The method of claim 1 wherein the telomerase is a mammalian telomerase.

4. The method of claim 1 wherein the telomerase is a human telomerase.

5. The method of claim 1 wherein the polynucleotide contains SEQ. ID NO:100.

6. The method of claim 1 further comprising comparing the sequence determined in step b) with the reverse transcriptase motif R-X^2-PK-X^4-R-X^1-I.

7. The method of claim 1 further comprising comparing the sequence determined in step b) with the reverse transcriptase motif F-X^3-D-X^3-CYD.

8. The method of claim 1 comprising deciding that the sample contains a polynucleotide sequence encoding at least a portion of telomerase if the sequence determined in step b) contains the amino acid motif

$$h_1\text{-}X^1\text{-}W\text{-}h_2\text{-}X^4\text{-}h_3\text{-}X^2\text{-}h_4\text{-}h_5\text{-}h_6\text{-}h_7\text{-}FFY\text{-}X^1\text{-}TE,$$

wherein

h_1 is L or I;

h_2 is L or;

h_3 is V or I;

h_4 is L or I;

h_5 is L or I;

h_6 is R or Q; and

h_7 is S, T or C.

* * * * *

US006921664B2

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,921,664 B2**
(45) **Date of Patent: *Jul. 26, 2005**

(54) **TELOMERASE**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Boulder, CO (US); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**, Davis, CA (US); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **Regents of the University of Colorado**, Boulder, CO (US); **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 271 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/054,295**

(22) Filed: **Jan. 18, 2002**

(65) **Prior Publication Data**

US 2003/0044953 A1 Mar. 6, 2003

Related U.S. Application Data

(63) Continuation of application No. 09/843,676, filed on Apr. 26, 2001, which is a continuation of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned.

(51) **Int. Cl.**[7] C12N 5/00; C12N 15/14; C12N 9/12; C12N 9/60; C12N 5/06

(52) **U.S. Cl.** 435/325; 435/320.1; 435/194; 435/224; 435/348; 435/252.3; 435/419

(58) **Field of Search** 435/194, 320.1, 435/325, 224.1, 348, 252.3, 419

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tancholtz et al.
3,850,752	A	11/1974	Schuurs et al.
3,939,350	A	2/1976	Kronick et al.
3,996,345	A	12/1976	Ullman et al.
4,275,149	A	6/1981	Litman et al.
4,277,437	A	7/1981	Maggio
4,366,241	A	12/1982	Tom et al.
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,816,567	A	3/1989	Cabilly et al.
4,965,188	A	10/1990	Mullis et al.
5,489,508	A	2/1996	West et al.
5,583,016	A	12/1996	Villeponteau et al.
5,597,697	A	1/1997	Diamond
5,747,317	A	5/1998	Cao
5,770,422	A	6/1998	Collins
5,917,025	A	6/1999	Collins
5,919,656	A	7/1999	Harrington et al.
6,093,809	A	7/2000	Cech et al.
6,166,178	A	12/2000	Cech et al.
6,258,535	B1	7/2001	Villeponteau et al.
6,261,556	B1	7/2001	Weinrich et al.
6,261,836	B1	7/2001	Cech et al.
6,309,867	B1	10/2001	Cech et al.
6,337,200	B1	1/2002	Morin
6,440,735	B1	8/2002	Gaeta
6,444,650	B1	9/2002	Cech et al.
6,475,789	B1	11/2002	Cech et al.
6,517,834	B1	2/2003	Weinrich et al.
6,608,188	B1	8/2003	Tsuchiya et al.
6,610,839	B1	8/2003	Cech et al.
6,617,110	B1	9/2003	Cech et al.
6,627,619	B2	9/2003	Cech et al.
2002/0164786	A1	11/2002	Cech et al.
2002/0187471	A1	12/2002	Cech et al.
2003/0009019	A1	1/2003	Cech et al.
2003/0032075	A1	2/2003	Cech et al.
2003/0059787	A1	3/2003	Cech et al.
2003/0096344	A1	5/2003	Cech et al.
2003/0100093	A1	5/2003	Cech et al.

OTHER PUBLICATIONS

Adams, Mark et al. "Initial Assessment of Human Gene Diversity and Expression Patterns Based Upon 83 Million Nucleotides of cDNA Sequence" *The Genome Directory: Supplement to Nature Sep. 28, 1995*, pp. 3–174, vol. 377, Issue 6547S.

U.S. Appl. No. 09/432,503, Cech et al.

U.S. Appl. No. 09/721,477, Cech et al.

U.S. Appl. No. 09/721,506, Cech et al.

1994 Genome Issue of *Science* (265:1981f).

Anderson and Young, "Quantitative Filter Hybridization" In *Nucleic Acid Hybridization* pp73–111 (1985).

Ausubal et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York NY (1989).

Autexier et al., "Reconstitution of human telomerase activity and identification of a minimal functional region of the human telomerase RNA," (1996) EMBO J, 15:5928.

Autexier, C. et al. "Telomerase and cancer: revisiting the telomere hypothesis", Trends in Biochemical Sciences, 10 (21): 387–391 (1996).

Auxexier, and Greider "Functional reconstitution of wild-type and mutant *Tetrahymena telomerase*," (1994) Genes Develop., 8:563.

(Continued)

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—Malgorzata A Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Scott L. Ausenhus

(57) **ABSTRACT**

The present invention is directed to expression vectors comprising a polynucleotide that encodes a human telomerase reverse transcriptase (hTRT) protein, variant, or fragment. The present invention is also directed to host cells that comprise expression vectors comprising a polynucleotide that encodes a hTRT protein variant, or fragment.

20 Claims, 78 Drawing Sheets

-continued

```
                885                 890                 895

Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu
            900                 905                 910

Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe
        915                 920                 925

Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
    930                 935                 940

Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945                 950                 955                 960

Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
                965                 970                 975

Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
            980                 985                 990

Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
        995                 1000                1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
    1010                1015                1020

Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

What is claimed is:

1. A recombinant expression vector containing a polynucleotide that comprises an encoding region for a telomerase reverse transcriptase protein, variant, or fragment,

wherein the protein, variant or fragment has telomerase catalytic activity when complexed with a telomerase RNA, and

wherein a single-stranded DNA consisting of said encoding region hybridizes to a second single-stranded DNA at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl, wherein said second DNA is exactly complementary to SEQ. ID NO:224, and T_m is the melting temperature under the same reaction conditions of double-stranded DNA having the sequence of SEQ. ID NO:224.

2. The expression vector of claim 1, further comprising a promoter, an enhancer, or a 3' untranslated region.

3. The expression vector of claim 1, selected from a recombinant bacteriophage, a plasmid, a cosmid, a yeast expression vector, and a viral expression vector.

4. The expression vector of claim 1, selected from a mammalian virus expression vector, an SV40 virus expression vector, an EBV expression vector, an *Autographa californica* nuclear polyhedrosis virus expression vector, an adenovirus expression vector, a retrovirus expression vector, a herpes virus expression vector, and a vaccinia virus expression vector.

5. The expression vector of claim 2, wherein the promoter is a constitutive promoter.

6. The expression vector of claim 2, wherein the promoter is an inducible promoter.

7. The expression vector of claim 2, wherein the promoter is selected from an alpha factor promoter, an alcohol oxidase promoter, a PGH promoter, a 35S promoter of CaMV, a 19S promoter at CaMV, a lacZ promoter, a ptrp-lac hybrid promoter, a polyhedrin promoter, a heat shock promoter, a RUBISCO promoter, and a storage protein gene promoter.

8. The expression vector of claim 1, further comprising a viral origin of replication.

9. The expression vector of claim 1, further comprising a selectable marker gene.

10. The expression vector of claim 9, wherein the selectable marker gene is selected from herpes simplex virus thymidine kinase, adenine phosphoribosyltransferase, dhfr, npt, ala, pat, trpB, hisD, anthocyanin, β-glucuronidase, and luciferase.

11. A host cell comprising the expression vector of claim 1.

12. A host cell comprising the expression vector of claim 2.

13. A host cell comprising the expression vector of claim 3.

14. A host cell comprising the expression vector of claim 4.

15. An expression vector containing a polynucleotide that comprises an encoding region for a polypeptide containing SEQ. ID NO:225, or fragment thereof that has telomerase catalytic activity when complexed with a telomerase RNA.

16. The expression vector of claim 15, which is an adenovirus expression vector, a retrovirus expression vector, a herpes virus expression vector, or a vaccinia virus expression vector.

17. The expression vector of claim 15, further comprising a constitutive or inducible promoter operably linked to the encoding region.

18. The expression vector of claim 15, which causes expression at telomerase reverse transcriptase in mammalian cells.

19. The expression vector of claim 15, in a composition that comprises a pharmaceutically acceptable carrier.

20. A host cell comprising the expression vector of claim 15.

* * * * *

US006927285B2

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 6,927,285 B2**
(45) **Date of Patent: *Aug. 9, 2005**

(54) **GENES FOR HUMAN TELOMERASE REVERSE TRANSCRIPTASE AND TELOMERASE VARIANTS**

(75) Inventors: **Thomas R. Cech**, Potomac, MD (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, San Diego, CA (US); **Karen B. Chapman**, Mill Valley, CA (US); **Gregg B. Morin**, Oakville (CA); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Reno, NV (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **University Technology Corporation**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/438,486**

(22) Filed: **Nov. 12, 1999**

(65) **Prior Publication Data**

US 2003/0009019 A1 Jan. 9, 2003

Related U.S. Application Data

(63) Continuation of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned.

(51) **Int. Cl.**[7] **C07H 21/04**; C12N 9/12; C12N 1/20; C12N 15/00; C07K 1/00

(52) **U.S. Cl.** **536/23.2**; 435/194; 435/252.3; 435/320.1; 530/350

(58) **Field of Search** 435/194, 252.3, 435/320.1; 530/350

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A		6/1974	Tanenholtz et al.
3,850,752	A		11/1974	Schuurs et al.
3,939,350	A		2/1976	Kronick et al.
3,996,345	A		12/1976	Ullman et al.
4,275,149	A		6/1981	Litman et al.
4,277,437	A		7/1981	Maggio
4,366,241	A		12/1982	Tom et al.
4,683,195	A		7/1987	Mullis et al.
4,683,202	A		7/1987	Mullis
4,816,567	A		3/1989	Cabilly et al.
4,965,188	A		10/1990	Mullis et al.
5,489,508	A		2/1996	West et al.
5,583,016	A	*	12/1996	Villeponteau et al. 435/91.3
5,597,697	A		1/1997	Diamond 435/6
5,747,317	A	*	5/1998	Cao 435/194
5,770,422	A		6/1998	Collins
5,917,025	A	*	6/1999	Collins et al. 536/23.2
5,919,656	A		7/1999	Harrington 435/69.1
6,093,809	A		7/2000	Cech et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2271718	A1	5/1998
GB	2 317 891	A	4/1998
JP	09154575	A	6/1997
WO	WO 93/23572		11/1993
WO	WO 94/17210	A1	8/1994
WO	WO 95/13382		5/1995
WO	WO 96/01835		1/1996
WO	WO 96/12811		5/1996
WO	WO 96/19580		6/1996
WO	WO 96/40868		12/1996
WO	WO 98/01542		1/1998
WO	WO 98/01543		1/1998
WO	WO 98/08938		2/1998
WO	WO 98/07838		3/1998
WO	WO 98/14592	A2	4/1998
WO	WO 98/14593	A2	4/1998
WO	WO 98/21343		5/1998
WO	WO 98/23759		6/1998
WO	WO 98/37181		8/1998
WO	WO 98/45450		10/1998
WO	WO98/59040		12/1998
WO	WO99/01560		1/1999
WO	WO 99/33998	A2	7/1999
WO	WO 99/38964	A2	8/1999
WO	WO 00/46355	A2	8/2000

OTHER PUBLICATIONS

Comparison of Applicants SEQ ID No : 173 and the Accession No. AA311750.*

1994 Genome Issue of *Science* (265:1981f).

Anderson and Young, "Quantitative Filter Hybridization" in *Nucleic Acid Hybridization* pp73–111 (1985).

Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York NY (1989).

Autexier et al., "Reconstitution of human telomerase activity and identification of a minimal functional region of the human telomerase RNA," (1996) *EMBO J*, 15:5928.

Auxexier and Greider, "Functional reconstitution of wild–type and mutant *Tetrahymena* telomerase," (1994) *Genes Develop.*, 8:563.

Autexier, Chantal, et al., Telomerase and cancer: revisiting the telomere hypothesis; Trends in Biochemical Sciences, 10 (21): 387–391 (1996).

(Continued)

Primary Examiner—Tekchand Saidha
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Townsend and Townsend and Crew LLP

(57) **ABSTRACT**

The present invention is directed to novel telomerase nucleic acids and amino acids. In particular, the present invention is directed to nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs, including the 123 kDa and 43 kDa telomerase protein subunits of *Euplotes aediculatus*, and related sequences from *Schizosaccharomyces*, *Saccharomyces* sequences, and human telomerase. The present invention is also directed to polypeptides comprising these telomerase protein subunits, as well as functional polypeptides and ribonucleoproteins that contain these subunits.

9 Claims, 71 Drawing Sheets

-continued

(2) INFORMATION FOR SEQ ID NO:223:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

Lys Lys Lys Lys Lys Lys Lys Lys
1 5

We claim:

1. An isolated cDNA encoding human telomerase protein, wherein said cDNA is contained in plasmid pGRN121 having ATCC Deposit Accession No. 209016.

2. An isolated cDNA encoding human telomerase reverse transcriptase protein, wherein the cDNA has the restriction map shown in FIG. 49.

3. An isolated nucleic acid encoding a naturally occurring human telomerase reverse transcriptase protein or variant thereof,

wherein the polyoucleotide hybridizes to a nucleic acid having the sequence in SEQ ID NO:173 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

4. An isolated cDNA encoding a naturally occurring human telomerase reverse transcriptase protein, wherein the 5' terminus of the CDNA consists of ATG covalently linked to a nucleotide sequence commencing with CCC GTC CCG (contained in SEQ. ID NO:173).

5. An isolated cDNA encoding human telomerase reverse transcriptase protein, wherein the cDNA hybridizes to the CDNA insert in plasmid pGRN121 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

6. An isolated cDNA encoding human telomerase reverse transcriptase protein, wherein the cDNA hybridizes to a nucleic acid having the sequence in SEQ ID NO:173 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

7. The isolated nucleic acid of claim 3, wherein the 5' terminus consists of ATG covalently linked to a nucleotide sequence commencing with CCC GTC CCG.

8. The nucleic acid of claim 3,

wherein the encoded human telomerase reverse transcriptase protein comprises the motifs FFYVTE (SEQ. ID NO:112), PKP, AYD, OG, and DD.

9. The nucleic acid of claim 3, which is a cDNA.

* * * * *

US007005262B2

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 7,005,262 B2**
(45) **Date of Patent: Feb. 28, 2006**

(54) **METHODS FOR DETECTING NUCLEIC ACIDS ENCODING HUMAN TELOMERASE REVERSE TRANSCRIPTASE**

(75) Inventors: **Thomas R. Cech**, Potomac, MD (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, San Diego, CA (US); **Karen B. Chapman**, Mil Valley, CA (US); **Gregg B. Morin**, Oakville (CA); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Reno, NV (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **The Regents of the University of Colorado**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 163 days.

(21) Appl. No.: **10/054,611**

(22) Filed: **Jan. 18, 2002**

(65) **Prior Publication Data**

US 2003/0059787 A1 Mar. 27, 2003

Related U.S. Application Data

(63) Continuation of application No. 09/843,676, filed on Apr. 26, 2001, which is a continuation of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned.

(51) **Int. Cl.**

C12Q 1/68	(2006.01)
C12N 9/12	(2006.01)
C07H 21/04	(2006.01)
C07H 21/02	(2006.01)

(52) **U.S. Cl.** 435/6; 435/194; 536/23.1; 536/23.2; 536/24.3; 536/24.31; 536/24.33

(58) **Field of Classification Search** 435/6, 435/194; 536/24.31, 23.1, 24.3, 23.2, 24.33
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tanenholtz et al.
3,850,752	A	11/1974	Schuurs et al.
3,939,350	A	2/1976	Kronick et al.
3,996,345	A	12/1976	Ullman et al.
4,275,149	A	6/1981	Litman et al.
4,277,437	A	7/1981	Maggio
4,366,241	A	12/1982	Tom et al.
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,816,567	A	3/1989	Cabilly et al.
4,965,188	A	10/1990	Mullis et al.
5,489,508	A	2/1996	West et al.
5,583,016	A	12/1996	Villeponteau et al.
5,597,697	A	1/1997	Diamond
5,747,317	A	5/1998	Cao
5,770,422	A	6/1998	Collins
5,917,025	A	6/1999	Collins
5,919,656	A	7/1999	Harrington et al.
6,093,809	A	7/2000	Cech et al.
6,166,178	A	12/2000	Cech et al.
6,258,535	B1	7/2001	Villeponteau et al.
6,261,556	B1	7/2001	Weinrich et al.
6,261,836	B1	7/2001	Cech et al.
6,309,867	B1	10/2001	Cech et al.
6,337,200	B1	1/2002	Morin
6,440,735	B1	8/2002	Gaeta
6,444,650	B1	9/2002	Cech et al.
6,475,789	B1	11/2002	Cech et al.
6,517,834	B1	2/2003	Weinrich et al.
6,608,188	B1	8/2003	Tsuchiya et al.
6,610,839	B1	8/2003	Morin et al.
6,617,110	B1	9/2003	Cech et al.
6,627,619	B1	9/2003	Cech et al.
2002/0164788	A1	11/2002	Cech et al.
2002/0187471	A1	12/2002	Cech et al.
2003/0009019	A1	1/2003	Cech et al.
2003/0032075	A1	2/2003	Cech et al.
2003/0044953	A1	3/2003	Cech et al.
2003/0096344	A1	5/2003	Cech et al.
2003/0100093	A1	5/2003	Cech et al.

FOREIGN PATENT DOCUMENTS

CA	2271718	A1	5/1998
GB	2 317 891	A	4/1998
JP	09154575	A	6/1997
WO	WO 84/03564	A1	9/1984
WO	WO 93/23572		11/1993
WO	WO 94/17210	A1	8/1994
WO	WO 95/13382		5/1995

(Continued)

OTHER PUBLICATIONS

Adams et al. Initial assessment of human gene diversity and expression patterns based on 83 million nucleotides of cDNA sequence, Nature 377 (6547 Suppl), 3-174, 1995.*

(Continued)

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—Malgorzata A. Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Townsend Townsend & Crew

(57) **ABSTRACT**

The present invention is directed to methods of identifying in a sample nucleic acids that encode human telomerase reverse transcriptase (hTRT) or its fragments. The present invention is also directed to oligonucleotide primers used in such methods. The invention is further directed to PCR products that hybridize under stringent conditions to a polynucleotide encoding hTRT, as well as hybridization complexes comprising one strand of a cellular hTRT nucleic acid and one strand of nucleic acid comprising a recombinant or synthetic fragment of hTRT.

40 Claims, 78 Drawing Sheets

-continued

```
Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

What is claimed is:

1. A method of identifying a nucleic acid that encodes human telomerase reverse transcriptase (hTRT) or fragment thereof in a sample, comprising:

a) combining the sample with a polynucleotide probe such that the probe hybridizes specifically to the nucleic acid if the nucleic acid encodes hTRT or fragment thereof;

b) detecting any hybrid formed as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the hybrid is detected;

wherein the probe hybridizes specifically to a DNA having the sequence of the hTRT encoding region of SEQ. ID NO:224 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl but does not hybridize to a DNA having the sequence of SEQ. ID NO:62 under the same reaction conditions;

wherein T_m is the melting temperature of double-stranded DNA having the sequence of said encoding region under the same reaction conditions.

2. A method of detecting a nucleic acid that encodes hTRT or fragment thereof in a sample, comprising:

a) combining the sample with a polynucleotide probe such that the probe hybridizes specifically to a nucleic acid comprising at least 100 consecutive nucleotides contained in SEQ. ID NO:224 it present in the sample:

b) detecting any hybrid formed as a result of a), and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the hybrid is detected;

wherein the polynucleotide probe consists essentially of a sequence identical or complementary to 25 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224 that are not contained in SEQ. ID NO:62.

3. The method of claim 2, wherein the hTRT nucleic acid is human genomic DNA.

4. The method of claim 2, wherein the hTRT nucleic acid is mRNA or cDNA.

5. The method of claim 2, wherein the hTRT nucleic acid consists essentially of 250 or more nucleotides of SEQ. ID NO:224.

6. The method of claim 2, wherein the hTRT nucleic acid consists essentially of 500 or more nucleotides of SEQ. ID NO:224.

7. The method of claim 2, wherein the probe consists essentially of a sequence identical or complementary to 30 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

8. The method of claim 2, wherein the probe consists essentially of a sequence identical or complementary to 50 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

9. The method of claim 2, wherein the probe consists essentially of a sequence identical or complementary to 100 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

10. A method of identifying a nucleic acid that encodes hTRT or fragment thereof in a sample, comprising:

a) combining the sample with a polynucleotide primer under conditions that the primer specifically primes amplification of SEQ. ID NO:224 or fragment thereof if present in the sample;

b) detecting any amplification product formed as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the amplification product is detected;

wherein the primer hybridizes specifically to a DNA having the sequence of the hTRT encoding region of SEQ. ID NO:224 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl, but does not hybridize to a DNA having the sequence of SEQ. ID NO:62 under the same reaction conditions;

wherein T_m is the melting temperature of double-stranded DNA having the sequence of said encoding region under the same reaction conditions.

11. A method of detecting a nucleic acid encoding hTRT or fragment thereof in a sample, comprising:

a) combining the sample with polynucleotide primers so as to prime amplification of nucleic acid encoding hTRT or fragment thereof if present in the sample;

b) detecting any amplified product formed as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the amplification product is detected:

wherein each of said primers consists essentially of a sequence identical or complementary to 15 or more consecutive nucleotides from the hTRT encoding

region of SEQ. ID NO:224, but at least one of the primers does not consist sequence identical or complementary to 15 or more consecutive nucleotides from SEQ. ID NO:62.

12. The method of claim 11, wherein each of said primers consists essentially of a sequence identical or complementary to 30 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

13. The method of claim 11, wherein each of said primers consists essentially of a sequence identical or complementary to 50 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

14. The method of claim 1, wherein a) comprises combining the sample with the probe at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

15. The method of claim 1, wherein the probe comprises a sequence identical or complementary to 100 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

16. The method of claim 1, wherein the sample has been taken from a patient having a tumor.

17. The method of claim 2, wherein the sample has been taken from a patient having a tumor.

18. The method of claim 10, wherein a) comprises combining the sample with the primer at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

19. The method of claim 10, wherein the primer comprises a sequence identical or complementary to 30 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224.

20. The method of claim 10, wherein the sample has been taken from a patient having a tumor.

21. The method of claim 11, wherein the sample has been taken from a patient having a tumor.

22. A method of identifying a nucleic acid that encodes human telomerase reverse transcriptase (hTRT) or fragment thereof in a sample, comprising:

a) combining the sample with a polynucleotide probe such that the probe hybridizes specifically to the nucleic acid if the nucleic acid encodes hTRT or fragment thereof;

b) detecting any hybrid formed as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the hybrid is detected;

wherein the probe hybridizes specifically to a DNA having a sequence consisting of SEQ. ID NO:62 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl;

wherein T_m is the melting temperature of double-stranded DNA having the sequence of SEQ. ID NO:62 under the same reaction conditions.

23. The method of claim 22, wherein a) comprises combining the sample with the probe at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

24. The method of claim 22, wherein the probe comprises a sequence identical or complementary to 100 or more consecutive nucleotides from SEQ. ID NO:62.

25. The method of claim 22, wherein the probe is a fragment of SEQ. ID NO:62.

26. The method of claim 22, wherein the sample has been taken from a patient having a tumor.

27. A method of detecting a nucleic acid that encodes hTRT or fragment thereof in a sample, comprising:

a) combining the sample with a polynucleotide probe such that the probe hybridizes specifically to a nucleic acid comprising at least 100 consecutive nucleotides contained in SEQ. ID NO:62 if present in the sample:

b) detecting any hybrid formed as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the hybrid is detected;

wherein the polynucleotide probe consists essentially of a sequence identical or complementary to 25 or more consecutive nucleotides from SEQ. ID NO:62.

28. The method of claim 27, wherein the probe consists essentially of a sequence identical or complementary to 30 or more consecutive nucleotides from SEQ. ID NO:62.

29. The method of claim 27, wherein the probe consists essentially of a sequence identical or complementary to 50 or more consecutive nucleotides from SEQ. ID NO:62.

30. The method of claim 27, wherein the probe consists essentially of a sequence identical or complementary to 100 or more consecutive nucleotides from SEQ. ID NO:62.

31. The method of claim 27, wherein the sample has been taken from a patient having a tumor.

32. A method of identifying a nucleic acid that encodes hTRT or fragment thereof in a sample, comprising:

a) combining the sample with a polynucleotide primer under conditions that the primer specifically primes amplification of SEQ. ID NO:62 or fragment thereof if present in the sample;

b) detecting any amplification product formed as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the amplification product is detected;

wherein the primer hybridizes specifically to a DNA having a sequence consisting of SEQ. ID NO:62 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl;

wherein T_m is the melting temperature of double-stranded DNA having the sequence of SEQ. ID NO:62 under the same reaction conditions.

33. The method of claim 32, wherein a) comprises combining the sample with the primer at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl.

34. The method of claim 32, wherein the primer comprises a sequence identical or complementary to 30 or more consecutive nucleotides from SEQ. ID NO:62.

35. The method of claim 32, wherein the probe is a fragment of SEQ. ID NO:62.

36. The method of claim 32, wherein the sample has been taken from a patient having a tumor.

37. A method of detecting a nucleic acid encoding hTRT or fragment thereof in a sample, comprising:

a) combining the sample with polynucleotide primers so as to prime amplification of nucleic acid encoding hTRT or fragment thereof it present in the sample:

b) detecting any amplified product fanned as a result of a); and

c) identifying the nucleic acid as encoding hTRT or fragment thereof if the amplification product is detected;

wherein each of said primers consists essentially of a sequence identical or complementary to 15 or more consecutive nucleotides from SEQ. ID NO:62.

38. The method of claim 37, wherein each of said primers consists essentially of a sequence identical or complementary to 30 or more consecutive nucleotides from SEQ. ID NO:62.

39. The method of claim 37, wherein each of said primers consists essentially of a sequence identical or complementary to 50 or more consecutive nucleotides from SEQ. ID NO:62.

40. The method of claim 37, wherein the sample has been taken from a patient having a tumor.

* * * * *

US007056513B2

(12) **United States Patent**
Cech et al.

(10) **Patent No.: US 7,056,513 B2**
(45) **Date of Patent: Jun. 6, 2006**

(54) **TELOMERASE**

(75) Inventors: **Thomas R. Cech**, Boulder, CO (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, Boulder, CO (US); **Karen B. Chapman**, Sausalito, CA (US); **Gregg B. Morin**, Palo Alto, CA (US); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **Regents of the University of Colorado**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 778 days.

(21) Appl. No.: **09/843,676**

(22) Filed: **Apr. 26, 2001**

(65) **Prior Publication Data**

US 2002/0164786 A1 Nov. 7, 2002

Related U.S. Application Data

(63) Continuation of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned, which is a continuation-in-part of application No. 08/724,643, filed on Oct. 1, 1996, now abandoned.

(51) **Int. Cl.**

A61K 39/00	(2006.01)
A61K 38/51	(2006.01)
A61K 38/00	(2006.01)
C12N 9/12	(2006.01)
C07K 1/00	(2006.01)

(52) **U.S. Cl.** **424/185.1**; 424/94.5; 435/194; 530/300; 530/350; 530/324; 530/325; 530/326

(58) **Field of Classification Search** 435/194; 530/300, 350; 424/94.5, 185.1
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tanenholtz et al.
3,850,752	A	11/1974	Schuurs et al.
3,939,350	A	2/1976	Kronick et al.
3,996,345	A	12/1976	Ullman et al.
4,275,149	A	6/1981	Litman et al.
4,277,437	A	7/1981	Maggio
4,366,241	A	12/1982	Tom et al.
4,683,195	A	7/1987	Mullis et al.
4,683,202	A	7/1987	Mullis
4,816,567	A	3/1989	Cabilly et al.
4,965,188	A	10/1990	Mullis et al.
5,489,508	A	2/1996	West et al.
5,583,016	A	12/1996	Villeponteau et al.
5,597,697	A	1/1997	Diamond
5,747,317	A	5/1998	Cao
5,770,422	A	6/1998	Collins
5,917,025	A	6/1999	Collins
5,919,656	A	7/1999	Harrington et al.
6,093,809	A	7/2000	Cech et al.
6,166,178	A	12/2000	Cech et al.
6,258,535	B1	7/2001	Villeponteau et al.
6,261,556	B1	7/2001	Weinrich et al.
6,261,836	B1	7/2001	Cech et al.
6,309,867	B1	10/2001	Cech et al.
6,337,200	B1	1/2002	Morin
6,440,735	B1 *	8/2002	Gaeta 435/372.2
6,444,650	B1	9/2002	Cech et al.
6,475,789	B1	11/2002	Cech et al.
6,517,834	B1	2/2003	Weinrich et al.
6,608,188	B1	8/2003	Tsuchiya et al.
6,610,839	B1	8/2003	Morin et al.
6,617,110	B1	9/2003	Cech et al.
6,627,619	B1	9/2003	Cech et al.
2002/0187471	A1	12/2002	Cech et al.
2003/0009019	A1	1/2003	Cech et al.
2003/0032075	A1	2/2003	Cech et al.
2003/0044953	A1	3/2003	Cech et al.
2003/0059787	A1	3/2003	Cech et al.
2003/0096344	A1	5/2003	Cech et al.
2003/0100093	A1	5/2003	Cech et al.

FOREIGN PATENT DOCUMENTS

CA	2271718	A1	5/1998
GB	2 317 891	A	4/1998
JP	09154575	A	6/1997
WO	WO 84/03564	A1	9/1984
WO	WO 93/23572		11/1993
WO	WO 94/17210	A1	8/1994
WO	WO 95/13382		5/1995

(Continued)

OTHER PUBLICATIONS

Adamson, D. et al. "Significant Telomere Shortening in Childhood Leukemia", Cancer Genet. Cytogenet, 1992; pp. 204–206, vol. 61.

(Continued)

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—Malgorzata A. Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; Scott L. Ausenhus; Townsend and Townsend and Crew LLP

(57) **ABSTRACT**

The present invention is directed to novel telomerase nucleic acids and amino acids. In particular, the present invention is directed to nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs, including the 123 kDa and 43 kDa telomerase protein subunits of *Euplotes aediculatus*, and related sequences from *Schizosaccharomyces*, *Saccharomyces* sequences, and human telomerase. The present invention is also directed to polypeptides comprising these telomerase protein subunits, as well as functional polypeptides and ribonucleoproteins that contain these subunits.

20 Claims, 78 Drawing Sheets

-continued

```
Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
    930                 935                 940

Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945                 950                 955                 960

Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
                965                 970                 975

Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
            980                 985                 990

Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
        995                 1000                1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
    1010                1015                1020

Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

We claim:

1. An isolated polypeptide that induces anti-hTRT specific antibody, consisting of 10 or more consecutive amino acids of SEQ. ID NO:225.

2. The polypeptide of claim 1, containing an amino acid sequence selected from SEQ. ID NO:112, SEQ. ID NO:113, SEQ. ID NO:114, SEQ. ID NO:115, SEQ. ID NO:116, and SEQ. ID NO;117.

3. The polypeptide of claim 1, which does not retain the telomerase catalytic activity of native human telomerase reverse transcriptase.

4. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

5. An immunogenic composition that induces anti-hTRT specific antibody, comprising a peptide and an adjuvant, wherein the peptide consists of 10 or more consecutive amino acids of SEQ. ID NO:225.

6. The composition of claim 5, wherein the adjuvant is selected from Freund's adjuvant, an mineral gel, aluminum hydroxide, lysolecithin, pluronic polyol, a polyanlon, a peptide, an oil emulsion, keyhole limpet hemocyanin (KLH), dinitrophenol (DNP), *Bacillus* Calmette-Guerin, and *Corynebacterium parvum*.

7. A method for eliciting an immune response to telomerase reverse transcriptase protein in a subject, comprising administering to the subject the composition of claim 5.

8. The method of claim 7, further comprising assessing whether telomerase-specific antibody is produced as a result of the administration.

9. An immunogenic composition that induces anti-hTRT specific antibody, comprising a peptide and an adjuvant, wherein the peptide consists of 5 to 10 consecutive amino acids of SEQ. ID NO:225.

10. The composition of claim 9, wherein the adjuvant is selected from Freund's adjuvant, an mineral gel, aluminum hydroxide, lysolecithin pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin (KLH), dinitrophenol (DNP), *Bacillus* Calmette-Guerin, and *Corynebacterium parvum*.

11. A method for eliciting an immune response to telomerase reverse transcriptase protein in a subject, comprising administering to the subject the composition of claim 9.

12. The method of claim 7, further comprising assessing whether telomerase-specific antibody is produced as a result of the administration.

13. The polypeptide of claim 1, produced by recombinant expression.

14. The polypeptide of claim 1, produced by chemical synthesis.

15. A chimeric molecule comprising:

a polypeptide that consists of 10 or more consecutive amino acids of SEQ. ID NO:225, and

an immunogenic second protein,

wherein the polypeptide is fused to the second protein so as to form a chimeric molecule that induces anti-hTRT specific antibody.

16. The chimeric protein of claim 15, wherein the second protein is keyhole limpet hemocyanin.

17. An immunogenic composition comprising the chimeric protein of claim 15, and an adjuvant.

18. A chimeric molecule comprising:

a polypeptide that consists of 5 to 10 consecutive amino acids of SEQ. ID NO:225, and

an immunogenic second protein,

wherein the polypeptide is fused to the second protein so as to form a chimeric molecule that induces anti-hTRT specific antibody.

19. The chimeric protein of claim 18, wherein the second protein is keyhole limpet hemocyanin.

20. An immunogenic composition comprising the chimeric protein of claim 18, and an adjuvant.

* * * * *

US007195911B2

(12) United States Patent
Cech et al.

(10) Patent No.: US 7,195,911 B2
(45) Date of Patent: *Mar. 27, 2007

(54) MAMMALIAN CELLS THAT HAVE INCREASED PROLIFERATIVE CAPACITY

(75) Inventors: **Thomas R. Cech**, Potomac, MD (US); **Joachim Lingner**, Epalinges (CH); **Toru Nakamura**, San Diego, CA (US); **Karen B. Chapman**, Southborough, MA (US); **Gregg B. Morin**, Oakville (CA); **Calvin B. Harley**, Palo Alto, CA (US); **William H. Andrews**, Reno, NV (US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **The Regents of the University of Colorado**, Boulder, CO (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 464 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/044,539**

(22) Filed: **Jan. 11, 2002**

(65) **Prior Publication Data**

US 2003/0100093 A1 May 29, 2003

Related U.S. Application Data

(63) Continuation of application No. 08/912,951, filed on Aug. 14, 1997, now Pat. No. 6,475,789, which is a continuation-in-part of application No. 08/854,050, filed on May 9, 1997, now Pat. No. 6,261,836, which is a continuation-in-part of application No. 08/851,843, filed on May 6, 1997, now Pat. No. 6,093,809, which is a continuation-in-part of application No. 08/846,017, filed on Apr. 25, 1997, now abandoned, which is a continuation-in-part of application No. 08/844,419, filed on Apr. 18, 1997, now abandoned.

(51) **Int. Cl.**
C12N 15/85 (2006.01)
C12N 15/11 (2006.01)

(52) **U.S. Cl.** 435/325; 536/23.1

(58) **Field of Classification Search** 435/455, 435/325

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Tanenholtz et al.	
3,850,752	A	11/1974	Schuurs et al.	
3,939,350	A	2/1976	Kronick et al.	
3,996,345	A	12/1976	Ullman et al.	
4,275,149	A	6/1981	Litman et al.	
4,277,437	A	7/1981	Maggio	
4,366,241	A	12/1982	Tom et al.	
4,683,195	A	7/1987	Mullis et al.	
4,683,202	A	7/1987	Mullis	
4,816,567	A	3/1989	Cabilly et al.	
4,965,168	A	10/1990	Yoshida et al.	
5,489,508	A	2/1996	West et al.	
5,583,016	A	12/1996	Villeponteau et al.	
5,597,697	A	1/1997	Diamond	
5,747,317	A	5/1998	Cao	
5,770,422	A	6/1998	Collins	
5,917,025	A	6/1999	Collins	
6,093,809	A	7/2000	Cech et al.	
6,166,178	A	12/2000	Cech et al.	
6,258,535	B1	7/2001	Villeponteau et al.	
6,261,556	B1	7/2001	Weinrich et al.	
6,261,836	B1	7/2001	Cech et al.	
6,309,867	B1	10/2001	Cech et al.	
6,337,200	B1	1/2002	Morin	
6,440,735	B1	8/2002	Gaeta	
6,444,650	B1	9/2002	Cech et al.	
6,475,789	B1 *	11/2002	Cech et al.	435/366
6,517,834	B1	2/2003	Weinrich et al.	
6,608,188	B1	8/2003	Tsuchiya et al.	
6,610,839	B1	8/2003	Morin et al.	
6,617,110	B1	9/2003	Cech et al.	
6,627,619	B2	9/2003	Cech et al.	
2002/0164786	A1	11/2002	Cech et al.	
2002/0187471	A1	12/2002	Cech et al.	
2003/0009019	A1	1/2003	Cech et al.	
2003/0032075	A1	2/2003	Cech et al.	
2003/0044953	A1	3/2003	Cech et al.	
2003/0059787	A1	3/2003	Cech et al.	
2003/0096344	A1	5/2003	Cech et al.	

FOREIGN PATENT DOCUMENTS

CA	2271718	A1	5/1998

(Continued)

OTHER PUBLICATIONS

Gearhart, J. New Potential for Human Embryonic Stem Cells (1998) Science 282:1061-1062.*

Thomson et al. Embryonic Stem Cell Lines Drived from Human Blastocysts. (1998) Science 282:1145-1147.*

Campbell et al., Totipotentcyor mulitpotentiality of cultured cells: Aplications and Prgress. (1997) Theriogenology 47:63-72.*

Bodnar et al. (1998) Extension of Life-Span by Introduction of Telomerase into Normal Human Cells. Science 279:349-352.*

Adamson, D. et al. "Significant Telomere Shortening in Childhood Leukemia", *Cancer Genet. Cytogenet*, 1992: pp. 204-206, vol. 61.

Autexier, Chantal, et al., Telomerase and cancer: revisiting the telomere hypothesis; Trends in Biochemical Sciences, 1996, pp. 387-391, vol. 10, No. 21.

(Continued)

Primary Examiner—Deborah Crouch
Assistant Examiner—Louis D Lieto
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp; Ted Apple

(57) **ABSTRACT**

The present invention is directed to cells comprising a recombinant polynucleotide sequence that encodes a telomerase reverse transcriptase protein, variant, or fragment having telomerase catalytic activity when complexed with a telomerase RNA.

38 Claims, 34 Drawing Sheets

-continued

1395 1400 1405

(2) INFORMATION FOR SEQ ID NO: 335:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: <Unknown>
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 335:

Gly Ser Thr His Ile Ser His Ile Ser His Ile Ser His Ile Ser His
1 5 10 15

Ile Ser His Ile Ser His Ile Ser His Ile Ser
20 25

What is claimed is:

1. An isolated mammalian cell comprising a recombinant polynucleotide containing a nucleic acid sequence that encodes a telomerase reverse transcriptase protein having telomerase catalytic activity when complexed with a telomerase RNA,

wherein the polynucleotide hybridizes to DNA having a sequence complementary to SEQ. ID NO:1 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl;

wherein T_m is the melting temperature at the same reaction conditions of double-stranded DNA having a sequence that consists of the full length of SEQ. ID NO:1; and

wherein the expression of the protein from the recombinant polynucleotide in the cell increases proliferative capacity of the cell.

2. The cell of claim 1, which is a human cell.

3. The cell of claim 1, wherein the recombinant polynucleotide contains a nucleic acid sequence that encodes SEQ. ID NO:2, or fragment thereof having telomerase catalytic activity when complexed with a telomerase RNA.

4. The cell of claim 3, wherein the recombinant polynucleotide contains SEQ. ID NO:1, or fragment thereof that encodes a protein having telomerase catalytic activity when complexed with a telomerase RNA.

5. The cell of claim 2, wherein the polynucleotlde encodes a full-length telomerase reverse transcriptase.

6. The cell of claim 2, wherein the polynucleotide encodes a human telomerase reverse transcriptase having the amino acid sequence of SEQ ID NO:2.

7. The cell of claim 2, which further comprises a selectable marker gene.

8. The cell of claim 2, wherein the recombinant polynucleotide comprises a constitutive promoter.

9. The cell of claim 2, wherein the recombinant polynucleotide comprises an inducible promoter.

10. The cell of claim 2, which is a liver cell.

11. The cell of claim 10, which is a hepatocyte.

12. The cell of claim 2, which is a nerve cell.

13. The cell of claim 12, which is a glial cell, astrocyte, or oligodendrocyte.

14. The cell of claim 12, which is a neuron of the central nervous system.

15. The cell of claim 14, which is a cholinergic or adrenergic cell.

16. The cell of claim 2, which is a retinal pigmented epithelial cell.

17. The cell of claim 2, which is a contractile cell.

18. The cell of claim 17, which is a heart muscle cell or smooth muscle cell.

19. The cell of claim 2, which is a fat cell.

20. The cell of claim 2, which is a fibroblast.

21. The cell of claim 2, which is a vascular endothelial cell.

22. The cell of claim 2, which is a hormone secreting cell.

23. The cell of claim 22, wherein the cell secretes insulin or glucagon.

24. The cell of claim 22, which is a pituitary cell, thyroid hormone secreting cell, or adrenal cell.

25. The cell of claim 2, which is a fat storing cell.

26. The cell of claim 2, which is an epithelial or mucosal cell.

27. The cell of claim 26, which is an oral cavity cell, stomach cell, or intestinal cell.

28. The cell of claim 26, which is a mammary gland, uterus, or prostate cell.

29. The cell of claim 26, which is an air space epithelial cell of the lung.

30. The cell of claim 2, which is a tubular cell of the kidney.

31. The cell of claim 2, which is a blood cell or a cell of the immune system.

32. The cell of claim 31, which is a T or B lymphocyte.

33. The cell of claim 31, which is a mast cell or eosinophil.

34. The cell of claim 31, which is a monocyte or macrophage.

35. The cell of claim 2, which is an osteoblast, osteocyte, or osteoclast.

36. The cell of claim 2, which is a chondrocyte or sinovial cell.

37. The cell of claim 2, which is a stem cell.

38. The cell of claim 37, which is an adult stem cell.

* * * * *

US007091021B2

(12) **United States Patent**
Morin

(10) **Patent No.: US 7,091,021 B2**
(45) **Date of Patent: Aug. 15, 2006**

(54) **INACTIVE VARIANTS OF THE HUMAN TELOMERASE CATALYTIC SUBUNIT**

(75) Inventor: **Gregg B. Morin**, Davis, CA (US)

(73) Assignee: **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 462 days.

(21) Appl. No.: **09/990,080**

(22) Filed: **Nov. 21, 2001**

(65) **Prior Publication Data**

US 2002/0102686 A1 Aug. 1, 2002

Related U.S. Application Data

(63) Continuation of application No. 09/128,354, filed on Aug. 3, 1998, now Pat. No. 6,337,200, which is a continuation-in-part of application No. 09/052,864, filed on Mar. 31, 1998, now abandoned.

(51) **Int. Cl.**

C12N 9/12	(2006.01)
C07K 1/00	(2006.01)
A61K 38/00	(2006.01)
A61K 38/51	(2006.01)
C07H 21/04	(2006.01)

(52) **U.S. Cl.** 435/194; 530/350; 514/12; 424/94.5; 536/23.5

(58) **Field of Classification Search** 435/194; 530/350, 300, 324, 327, 388.21
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,261,556 B1	7/2001	Weinrich et al.	424/94.5
6,261,836 B1	7/2001	Cech et al.	435/325
6,846,662 B1 *	1/2005	Kilian et al.	435/194
6,916,642 B1 *	7/2005	Kilian et al.	435/194

FOREIGN PATENT DOCUMENTS

WO	WO 98/07838	2/1998
WO	WO 98/21343	5/1998
WO	WO 98/37181	8/1998
WO	WO 99/01560	1/1999

OTHER PUBLICATIONS

Molecular Biology and Biotechnology. A Comprehensive Desk Reference, Meyers R. ed. Wiley-VCH, New York 1995, p. 187.*

Leem et al., The human telomerase gene: complete genomic sequence and analysis of tandem repeat polymorphisms in intronic regions, Oncogene, 2002, 21, 769-777.*

Li H. et al. Protein Phosphatase 2A Inhibits Nuclear Telomerase Activity in Human Brest Cancer Cells, J. Biol. Chem, 1997, 272, 16729-16732.*

Bachand et al., Functional Regions of Human Telomerse Reverse Transcriptase and Human Telomerase RNA Required for Telomerase Activity and RNA-Protein Interactions, Mol. and Cellular Biol. 21:1888 (2001).

Bodnar et al., Extension of Life-span by Induction of Telomerase into Normal Human Cells, Science 279:349 (1998).

Bryan et al., A Mutant of *Tetrahymena* Telomerase Reverse Transcriptase with Increased Processivity, J. Biol. Chem. 275:24199 (2000).

Bryan et al., Telomerase RNA Bound by Protein Notifs Specific to Telomerase Reverse Transcriptase, Molecular Cell 6:493 (2000).

Bryan et al., Telomerase reverse transcriptase genes identified in *Tetrahymena thermophila* and *Oxytricha trifallax*, Pro. Natl. Acad. Sci. USA 95:8479 (1998).

Colgin et al., The hTERTapha splice variant is a dominant negative inhibitor of telomerase activity, Neoplasia 2:426 (2000).

Farmery et al., Major Histocompatability Class I Folding, Assembly, and Degradation: A Paradigm for Two-Stage Quality Control in the Endoplasmic Reticulum, Progress in Nucleic Acid Res. 67:235 (2001).

Freidman et al., Essential functions of amino-terminal domains in the yeast telomerase catalytic subunit revealed by selection for variable mutants, Genes & Dev. 13:2863 (1999).

Haering et al., Analysis of telomerase catalytic subunit mutants *in vivo* and *In vitro* in *Schizosaccharomyces pombe*, PNAS 97:6367 (2000).

Hahn et al., Inhibition of telomerase limits the growth of human cancer cells, Nature Medicine 5:1164 (1999).

Harrington et al., Human telomerase contians evolutionarily consderved catalytic and structural subunits, Genes Dev. 11:3109 (1997).

Killan et al., Isolation of a Candidate Human Telomerase Catalytic Subunit Gene, Which Reveals Complex Splicing Patterns in Different Cell Types, Hum. Mol. Genet. 6:2011 (1997).

Lai et al., RNA Binding Domain of Telomerase Reverse Transcriptase, Mol. and Cellular Biol. 21:990 (2001).

Lingner et al., Reverse Transcriptase Motifs in the Catalytic Subunit of Telomerase, Science 276:561 (1997).

Morin, The Implications of Telomerase Biochemistry for Human Disease, Eur. J. Biol. Chem. 33:750(1998).

Myerson et al., hEST2, the Putative Human Telomerase Catalytic Subunit Gene Is Up-Regulated in Tumor Cells and during Immortalization, Cell 90:785 (1997).

Nakamura et al., Telomerase Catalytic Subunit Homologs from Fission Yeast and Human, Science 277:955 (1997).

Perez et al., Human formyl peptide receptor ligand binding domain(s). Studies using an improved mutagenesis/expression vector reveal a novel mechanism for the regulation of receptor occupancy, J. Biol. Chem. 269:22485 (1994).

(Continued)

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—Malgorzata A. Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp

(57) **ABSTRACT**

The invention provides compositions and methods related to human telomerase reverse transcriptase (hTRT), the catalytic protein subunit of human telomerase. Catalytically active and inactive human telomerase reverse transcriptase variants comprising deletions or other mutations are provided.

11 Claims, 2 Drawing Sheets

-continued

<210> SEQ ID NO 20
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:RT6 oligo

<400> SEQUENCE: 20

gacgggctgc ggccgattgt gaacatggac ctgttcagcg tgctcaacta cgagcgggcg 60

<210> SEQ ID NO 21
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:RT8 oligo

<400> SEQUENCE: 21

acgtactgcg tgcgtcggta tgccgtggtc accttgacag acctccagcc gtacatgcga 60

What is claimed is:

1. A polypeptide encoded by DNA that hybridizes to the sequence complementary to SEQ. ID NO:1 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl,

wherein T_m is the melting temperature of double-stranded DNA having the sequence of SEQ. ID NO:1 under the same reaction conditions;

wherein said polypeptide has one or more of the following deletions:

a) residues 560–565,

b) residues 930–934,

c) at least 10 consecutive amino acids from residues 326–415,

d) at least 10 consecutive amino acids from residues 637–660,

e) at least 10 consecutive amino acids from residues 748–766,

f) at least 10 consecutive amino acids from residues 1055–1071, or

g) at least 10 consecutive amino acids from residues 1084–1116 of SEQ. ID NO:2;

and wherein said polypeptide inhibits telomerase enzyme activity when introduced into a cell expressing human telomerase reverse transcriptase (hTRT) (SEQ. ID NO:2).

2. A polypeptide lacking telomerase enzyme activity, wherein said polypeptide comprises full-length hTRT (SEQ ID NO: 2), except for one or more deletions(s) selected from the group consisting of:

a) residues 560–565,

b) residues 930–934,

c) at least 10 consecutive amino acids between residues 323–450,

d) at least 10 consecutive amino acids between residues 637–660,

e) at least 10 consecutive amino acids between residues 748–766,

f) at least 10 consecutive amino acids between residues 1055–1071, or

g) at least 10 consecutive amino acids between residues 1084–1116.

3. A polypeptide lacking telomerase enzyme activity, wherein said polypeptide comprises full-length hTRT (SEQ. ID NO:2), except for one or more deletions(s) consisting essentially of residues 560–565, 930–934, 326–415, 637–660, 748–766, 1055–1071, or 1084–1116,

wherein said polypeptide lacks telomerase catalytic activity;

and wherein said polypeptide inhibits telomerase enzyme activity when introduced into a cell expressing hTRT.

4. A method of inhibiting telomerase catalytic activity, comprising introducing a polypeptide according to claim 1 into an environment containing telomerase reverse transcriptase.

5. A method of inhibiting telomerase catalytic activity in a cell, comprising expressing in the cell a nucleic acid encoding a polypeptide according to claim 1.

6. A method of inhibiting telomerase catalytic activity, comprising introducing a polypeptide according to claim 2 into an environment containing telomerase reverse transcriptase.

7. A method of inhibiting telomerase catalytic activity in a cell, comprising expressing in the cell a nucleic acid encoding a polypeptide according to claim 2.

8. A method of producing an inactive variant of telomerase reverse transcriptase in a cell, comprising transfecting the cell to express a polypeptide according to claim 2.

9. A method of inhibiting telomerase catalytic activity, comprising introducing a polypeptide according to claim 3 into an environment containing telomerase reverse transciptase.

10. A method of inhibiting telomerase catalytic activity in a cell, comprising expressing in the cell a nucleic add encoding a polypeptide according to claim 3.

11. A method of producing an inactive variant of telomerase reverse transcriptase in a cell, comprising transfecting the cell to express a polypeptide according to claim 3.

* * * * *

US006337200B1

(12) **United States Patent**
Morin

(10) **Patent No.: US 6,337,200 B1**
(45) **Date of Patent: Jan. 8, 2002**

(54) **HUMAN TELOMERASE CATALYTIC SUBUNIT VARIANTS**

(75) Inventor: **Gregg B. Morin**, Palo Alto, CA (US)

(73) Assignee: **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/128,354

(22) Filed: **Aug. 3, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/052,864, filed on Mar. 31, 1998, now abandoned.

(51) **Int. Cl.**[7] **C07H 21/04**; C07K 1/00; C12N 5/00; C12N 15/63; C12N 15/85

(52) **U.S. Cl.** **435/194**; 435/69.1; 435/70.1; 435/320.1; 435/325; 435/440; 435/455; 514/44; 530/350; 536/23.1; 536/23.5

(58) **Field of Search** 536/23.5, 23.1, 536/24.5; 435/69.1, 325, 70.1, 71.1, 320.1, 440, 455; 514/44; 530/350

(56) References Cited

PUBLICATIONS

Bodnar et al., "Extension of Life-Span by Introduction of Telomerase into Normal Human Cells" *Science 279:* 349–352.

Harrington et al., 1997, "Human telomerase contains evolutionarily conserved catalytic and structural subunits-" *Genes Dev. 11:* 3109–3115.

Kilian et al., 1997, "Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types" *Hum. Mol. Genet. 6:* 2011–2019.

Meyerson et al., 1997, "hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization" *Cell 90:* 785–795.

Nakamura et al., 1997, "Telomerase Catalytic Subunit Homologs from Fission Yeast and Human" *Science 277:* 955.

Weinrich et al., 1997, "Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT" *Nat. Genet. 1997 Dec. 1:* 17(4):498–502.

Primary Examiner—Deborah Crouch
Assistant Examiner—Anne-Marie Baker
(74) *Attorney, Agent, or Firm*—Geron Corporation; J. Michael Schiff; David J. Earp

(57) ABSTRACT

The invention provides compositions and methods related to human telomerase reverse transcriptase (hTRT), the catalytic protein subunit of human telomerase. Catalytically active human telomerase reverse transcriptase variants comprising deletions or other mutations are provided.

11 Claims, 2 Drawing Sheets

-continued

<400> SEQUENCE: 21

acgtactgcg tgcgtcggta tgccgtggtc accttgacag acctccagcc gtacatgcga 60

What is claimed is:

1. A polynucleotide encoding a variant of human telomerase reverse transcriptase (hTRT), said variant having processive catalytic activity and comprising a deletion of at least 10 amino acids from region 192–323 or 415–450 of SEQ. ID NO:2.

2. The polynucleotide of claim 1, wherein the variant comprises a deletion of at least 25 amino acids from region 192–323 or 415–450 of SEQ. ID NO:2.

3. The polynucleotide of claim 1, further comprising a promoter sequence operably linked to the nucleotide sequence encoding the hTRT variant.

4. The polynucleotide of claim 1 that has a deletion of at least one region encoding exactly amino acids 192–323, 200–323, 200–271, 222–240, or 415–450 of SEQ. ID NO:2.

5. The polynucleotide of claim 1 that does not comprise a deletion in the region encoding amino acids 415–450.

6. The polynucleotide of claim 5, further comprising a promoter sequence operably linked to the nucleotide sequence encoding the hTRT variant.

7. A method for increasing the proliferative capacity of a human cell in vitro, comprising expressing the polynucleotide of claim 6 in the cell, thereby increasing its proliferative capacity.

8. A method for increasing the proliferative capacity of a human cell in vitro, comprising expressing the polynucleotide of claim 3 in the cell, thereby increasing its proliferative capacity.

9. A method for producing a variant telomerase reverse transcriptase, comprising expressing the polynucleotide of claim 1 in a host cell or in a cell-free expression system.

10. A cell comprising the polynucleotide of claim 1.

11. The cell of claim 10, that is a human cell.

* * * * *

US006610839B1

(12) **United States Patent**
Morin et al.

(10) **Patent No.: US 6,610,839 B1**
(45) **Date of Patent: Aug. 26, 2003**

(54) **PROMOTER FOR TELOMERASE REVERSE TRANSCRIPTASE**

(75) Inventors: **Gregg B. Morin**, Davis, CA (US); **William H. Andrews**, Richmond, CA (US)

(73) Assignee: **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/402,181**

(22) PCT Filed: **Oct. 1, 1997**

(86) PCT No.: **PCT/US97/17885**

§ 371 (c)(1),
(2), (4) Date: **Sep. 29, 1999**

(87) PCT Pub. No.: **WO98/14593**

PCT Pub. Date: **Apr. 9, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/912,951, filed on Aug. 14, 1997, and a continuation-in-part of application No. 08/911,312, filed on Aug. 14, 1997, now abandoned, and a continuation-in-part of application No. 08/915,503, filed on Aug. 14, 1997, now abandoned.

(51) **Int. Cl.**[7] C07H 21/04; C12N 9/12; C12N 15/00

(52) **U.S. Cl.** 536/24.1; 435/194; 435/320.1

(58) **Field of Search** 435/194, 320.1; 536/24.1

(56) References Cited

U.S. PATENT DOCUMENTS

3,817,837	A	6/1974	Rubenstein et al.	
3,850,752	A	11/1974	Schuurs et al.	
3,939,350	A	2/1976	Kronick et al.	
3,996,345	A	12/1976	Ullman et al.	
4,275,149	A	6/1981	Litman et al.	
4,277,437	A	7/1981	Maggio	
4,366,241	A	12/1982	Tom et al.	
4,683,195	A	7/1987	Mullis et al.	
4,683,202	A	7/1987	Mullis	
4,816,567	A	3/1989	Cabilly et al.	
4,965,188	A	10/1990	Mullis et al.	
5,416,017	A	5/1995	Burton et al.	
5,489,508	A	2/1996	West et al.	
5,583,016	A	12/1996	Villeponteau et al.	
5,631,236	A	5/1997	Woo et al.	
5,728,379	A	3/1998	Martuza et al.	
5,747,317	A	5/1998	Cao	
5,770,422	A	6/1998	Collins	
5,907,083	A	5/1999	Robert et al.	800/205
5,998,205	A	12/1999	Hallenbeck et al.	
6,054,575	A	4/2000	Villeponteau et al.	536/24.31
6,083,717	A	7/2000	Madzak et al.	435/69.1
6,093,809	A	7/2000	Cech et al.	
6,175,060	B1	1/2001	Lefebvre et al.	800/295
6,228,643	B1	5/2001	Greenland et al.	435/419
6,261,556	B1	7/2001	Weinrich et al.	424/94.5
6,261,836	B1	7/2001	Cech et al.	435/325
6,271,437	B1	8/2001	Jessen et al.	800/278
6,274,790	B1	8/2001	Kunst et al.	800/287
6,281,409	B1	8/2001	Woodhead et al.	800/287
6,300,095	B1	10/2001	Barredo Fuente et al.	435/69.1
6,306,656	B1	10/2001	Liu et al.	435/419
6,331,527	B1	12/2001	Parmacek et al.	514/44

FOREIGN PATENT DOCUMENTS

GB	2317891	4/1998
JP	09154575 A	6/1997
WO	WO 84/03564	9/1984
WO	WO 95/13382	5/1995
WO	WO 96/01835	1/1996
WO	WO 96/12811	5/1996
WO	WO 96/19580	6/1996
WO	WO 96/40868	12/1996
WO	WO 98/01542	1/1998
WO	WO 98/01543	1/1998
WO	WO 98/07838	2/1998
WO	WO 98/08938	3/1998
WO	WO 98/14592	4/1998
WO	WO 98/14593	4/1998
WO	WO 98/21343	5/1998
WO	WO 98/37181	8/1998
WO	WO 98/45450	10/1998
WO	WO 98/59040	12/1998
WO	WO 99/01560	1/1999
WO	WO 99/33998	7/1999
WO	WO 99/38964	8/1999
WO	WO 00/46355	8/2000

OTHER PUBLICATIONS

Greenberg R. A. et al. Telomerase reverse transcriptase gene is a direct target of c–Myc but is not functionally equivalent in cellular transformation, Oncogene (1999) 18, 1219–1226.*

Takakura M. et al. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cell, Cancer Res. (1999) 59, 551–557.*

Wick M. et al. Genomic organization and promoter characterization of the gene encoding the human telomerase reverse transcriptase (hTERT), Gene (1999) 232, 97–106.*

Wu K.–J. et al, Direct activation of TERT transcription by c–MYC, Nature Gentet. (1999) 21, 220–224.*

(List continued on next page.)

Primary Examiner—Rebecca E. Prouty
Assistant Examiner—Malgorzata A. Walicka
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp

(57) **ABSTRACT**

The invention provides compositions and methods related to human telomerase reverse transcriptase (hTRT), the catalytic protein subunit of human telomerase. The polynucleotides and polypeptides of the invention are useful for diagnosis, prognosis and treatment of humana diseases, for changing the proliferative capacity of cells and organisms, and for identification and screening of compounds and treatments useful for treatment of diseases such as cancers.

34 Claims, 103 Drawing Sheets

What is claimed is:

1. An isolated nucleic acid comprising a promoter sequence that either:

a) is contained in lambda phage Gϕ5 deposited as ATCC Accession No. 98505; or

b) hybridizes to the DNA of lambda phage Gϕ5 at 5 to 25° C. below the melting temperature (T_m) of a double-stranded DNA having the sequence of lambda phage Gϕ5 in aqueous solution at 1 M NaCl;

wherein the promoter sequence promotes transcription in cells endogenously expressing human telomerase reverse transcriptase (hTRT).

2. An isolated nucleic acid comprising a promoter sequence that is at least 80% identical to the 1.8 kilobases of SEQ. ID NO:6 that are upstream of the translation initiation site;

wherein the promoter sequence promotes transcription in cells endogenously expressing human telomerase reverse transcriptase (hTRT).

3. The nucleic acid of claim 1, which hybridizes to lambda phage Gϕ5 at 5° C. below T_m in aqueous solution at 1 M NaCl.

4. The nucleic acid of claim 2, wherein the promoter sequence comprises at least 100 consecutive nucleotides that are at least 90% identical to a sequence contained in SEQ. ID NO:6 within 1.8 kilobases upstream of the translation initiation site.

5. The nucleic acid of claim 2, wherein the promoter sequence comprises at least 200 consecutive nucleotides that are at least 90% identical to a sequence contained in SEQ. ID NO:6 within 1.8 kilobases upstream of the translation initiation site.

6. The nucleic acid of claim 2, wherein the promoter sequence comprises the 1.8 kilobases of SEQ. ID NO:6 that are upstream of the translation initiation site.

7. The nucleic acid of claim 2, which is a DNA.

8. The nucleic acid of claim 2 contained in a viral vector.

9. The nucleic acid of claim 8, wherein the viral vector is an adenovirus vector or a retrovirus vector.

10. The nucleic acid of claim 2 contained in a host cell.

11. The nucleic acid of claim 2, in which the promoter sequence is operably linked to a heterologous sequence, such that the heterologous sequence is transcribed in cells endogenously expressing TRT.

12. The nucleic acid of claim 11, wherein the heterologous sequence is a reporter gene.

13. The nucleic acid of claim 12, wherein the reporter gene encodes a protein that is fluorescent, phosphorescent, or has enzymatic activity.

14. The nucleic acid of claim 11, wherein the heterologous sequence is a gene which, upon expression in a cell, is toxic to the cell.

15. The nudieic acid of claim 11, wherein the heterologous sequence is a gene which, upon expression in a cell, renders the cell more susceptible to toxicity of a drug.

16. The nucleic acid of claim 15, wherein the gene encodes thymidine kinase.

17. An isolated or recombinant nucleic acid comprising a promoter sequence containing the 1.8 kB of SEQ. ID NO:6 upstream of the transcription initiation site for human telomerase reverse transcriptase (hTRT), or a fragment thereof that promotes transcription in cells endogenously expressing hTRT.

18. The nucleic acid of claim 17, containing at least 100 consecutive nucleotides of SEQ. ID NO:6 within 1.8 kilobases upstream of the translation initiation site.

19. The nucleic acid of claim 17, containing at least 200 consecutive nucleotides of SEQ. ID NO:6 within 1.8 kilobases upstream of the translation initiation site.

20. The nucleic acid of claim 17, further comprising a sequence from within the first intron of SEQ. ID NO:6.

21. The nucleic acid of claim 17 contained in a viral vector.

22. The nucleic acid of claim 21, wherein the viral vector is an adenovirus vector or a retrovirus vector.

23. The nucleic acid of claim 17, in which the promoter sequence is operably linked to a heterologous sequence, such that the heterologous sequence is transcribed in cells endogenously expressing TRT.

24. The nucleic acid of claim 23, wherein the heterologous sequence is a reporter gene.

25. The nucleic acid of claim 24, wherein the reporter gene encodes a protein that is fluorescent, phosphorescent, or has enzymatic activity.

26. The nucleic acid of claim 23, wherein the heterologous sequence is a gene which, upon expression in a cell, is toxic to the cell.

27. The nucleic acid of claim 23, wherein the heterologous sequence is a gene which, upon expression in a cell, renders the cell more susceptible to toxicity of a drug.

28. The nucleic acid of claim 1 contained in a viral vector.

29. The nucleic acid of claim 28, wherein the viral vector is an adenovirus vector or a retrovirus vector.

30. The nucleic acid of claim 1, in which the promoter sequence is operably linked to a heterologous sequence, such that the heterologous sequence is transcribed in cells endogenously expressing TRT.

31. The nucleic acid of claim 30, wherein the heterologous sequence is a reporter gene.

32. The nucleic acid of claim 31, wherein the reporter gene encodes a protein that is fluorescent, phosphorescent, or has enzymatic activity.

33. The nucleic acid of claim 30, wherein the heterologous sequence is a gene which, upon expression in a cell, is toxic to the cell.

34. The nucleic acid of claim 30, wherein the heterologous sequence is a gene which, upon expression in a cell, renders the cell more susceptible to toxicity of a drug.

* * * * *

US006767719B1

(12) **United States Patent**
Morin et al.

(10) **Patent No.: US 6,767,719 B1**
(45) **Date of Patent: *Jul. 27, 2004**

(54) **MOUSE TELOMERASE REVERSE TRANSCRIPTASE**

(75) Inventors: **Gregg B. Morin**, Palo Alto, CA (US); **Richard Allsopp**, Mountain View, CA (US); **Ronald A. DePinho**, Pelham Manor, NY (US); **Roger A. Greenberg**, Bronx, NY.(US)

(73) Assignees: **Geron Corporation**, Menlo Park, CA (US); **Albert Einstein College of Medicine of Yeshiva University, a division of Yeshiva University**, Bronx, NY (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/042,460**

(22) Filed: **Mar. 16, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/979,742, filed on Nov. 26, 1997, now abandoned.

(51) **Int. Cl.**[7] **C12P 21/06**; C12N 5/00; C12N 15/63; C07H 21/04; C07K 1/00

(52) **U.S. Cl.** **435/69.1**; 435/320.1; 435/325; 435/455; 536/23.1; 536/23.5; 530/350

(58) **Field of Search** 435/69.1, 320.1, 435/325, 455; 536/23.1, 23.5, 23.7; 530/350; 800/3, 13, 18

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,337,200 B1	1/2002	Morin	435/194
2003/0060417 A1	3/2003	Tsuchiya et al.	514/12

FOREIGN PATENT DOCUMENTS

WO	WO/9735967	* 10/1997
WO	WO 97/35967	10/1997
WO	WO97/35967	* 10/1997
WO	PCT/US/98/25211	11/1998
WO	WO 99/35261	7/1999
WO	WO 02/74935	9/2002

OTHER PUBLICATIONS

Ngo, Computational complexity Protein structure prediction and the Levinthal paradox in The Protein Folding Problem and Tertiary Structure Prediction, Merz et al. (eds.), Birkhuaser Boston: Boston, MA, pp. 433 and 492–495, 1994).*

Rudinger Characteristics of amino acids as components of a peptide hormone sequence (in Peptide Hormones, Parsons (ed.), University Park Press: Baltimore, MD, pp. 1–7, 1976).*

PTO Sequence Search report ACC. NO. AF015950, Science 277:955–959, 1997.*

Rosenberg et al., Gene therapist, Heal thyself, 2000, Science, vol. 287, pp. 1751.*

Friedmann, Principles for human gene therapy studies, 2000, Science, vol. 287, pp. 2163–2164.*

Anderson, Human gene therapy, 1998,Nature, vol. 392, pp. 25–30.*

Verma et al., Gene therapy–promises,problems and prospects, 1997, Nature, vol. 389, pp. 239–242.*

Ngo, in The Protein Folding Problem and Tertiary Structure Prediction, Merz et al. (eds.), Birkhauser Boston: Boston, MA, pp. 433 and 492–495, 1994.*

Rudinger, in Peptide Hormones, Parsons (ed.), University Park Press: Baltimore, MD, pp. 1–7, 1976.*

Lundblad, PNAS 95:8415–8416, 1998.*

Rossant et al , Phil. Trans. R. Soc Lond. B. 339:137–254, 1993.*

PTO Sequence Search report ACC. NO. AF015950, Science 277:955–959, 1997.*

Nakamura et al.; *Telomerase Catalytic Subunit Homologs from Fission Yeast and Human*, Science vol. 277, Aug. 1997, pp. 955–959.

Meyerson et al. *Hest2 the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization*, Cell vol. 90, Aug. 1997, pp. 785–795.

Bryan TM, Sperger JM, Chapman KB, Cech TR. Telomerase reverse transcriptase genes identified in Tetrahymena thermophila and Oxytricha trifallax. Proc Natl Acad Sci U S A. Jul. 21, 1998; 95(15):8479–84.

Collins K, Gandhi L. The reverse transcriptase component of the Tetrahymena telomerase ribonucleoptroein complex. Proc Natl Acad Sci U S A . Jul. 21, 1998;95(15):8485–90.

Counter CM, Meyerson M, Eaton EN, Weinberg RA. The catalytic subunit of yeast telomerase. Proc Natl Acad Sci USA. Aug. 19, 1997;94(17):9202–7.

Friedman KL, Cech TR. Essential functions of amino-terminal domians in the yeast telomerase catalytic subunit revealed by selection for viable mutants. Genes Dev. Nov. 1, 1999;13(21):2863–74.

(List continued on next page.)

Primary Examiner—Jeffrey Fredman
Assistant Examiner—Sumesh Kaushal
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp

(57) **ABSTRACT**

This invention provides for murine telomerase reverse transcriptase (mTERT) enzyme proteins and nucleic acids, including methods for isolating and expressing these nucleic acids and proteins, which have application to the control of cell proliferation and aging, including the control of age-related diseases, such as cancer.

21 Claims, 16 Drawing Sheets-

-continued

1 5 10

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Leu Leu Arg Phe Xaa Asp Asp Phe Leu Leu Xaa Thr
1 5 10

What is claimed is:

1. An isolated, purified or recombinant polynucleotide encoding a telomerase reverse transcriptase protein, wherein said protein:

(i) has at least 90% sequence identity to SEQ. ID NO:2;

(ii) has telomerase catalytic activity when associated with telomerase RNA component; and

(iii) contains at least one of the following amino acid motifs;

Motif T: W-X_{12}-FFY-X_1-TE-X_{11}-R-X_3-W;

Motif 1: LR-X_1-IPK;

Motif 2: R-X_1-I-X_{15}-K;

Motif A: P-X_3-F-X_3-D-X_4-YD;

Motif B: Y-X_4-G-X_2-QG-X_3-S;

Motif C: DD-X_1-L; or

Motif D: A-X_2-F-X_{18}-K;

wherein X_n is a sequence of unspecified amino acids of length "n".

2. An isolated, purified or recombinant polynucleotide encoding a telomerase reverse transcriptase protein having the amino acid sequence of SEQ. ID NO:2.

3. An isolated, purified or recombinant polynucleotide comprising the sequence of SEQ. ID NO:1, or fragment thereof that encodes a protein having telomerase activity when associated with telomerase RNA component; wherein the protein contains at least one of the following amino acid motifs;

Motif T: W-X_{12}-FFY-X_1-TE-X_{11}-R-X_3-W;

Motif 1: LR-X_1-IPK;

Motif 2: R-X_1-I-X_{15}-K;

Motif A: P-X_3-F-X_3-D-X_4-YD;

Motif B: Y-X_4-G-X_2-QG-X_3-S;

Motif C: DD-X_1-L; or

Motif D: A-X_2-F-X_{18}-K;

wherein X_n is a sequence of unspecified amino acids of length "n".

4. An isolated cell transfected with the polynucleotide of claim 1, or progeny thereof.

5. An isolated cell transfected with the polynucleotide of claim 2, or progeny thereof.

6. An isolated cell transfected with the polynucleotide of claim 3, or progeny thereof.

7. An expression vector comprising the polynucleotide of claim 1.

8. An expression vector comprising the polynucleotide of claim 2.

9. The polynucleotide of claim 1, encoding a protein that is between about 50 and 150 kDa.

10. The polynucleotide of claim 1, encoding a protein that contains Motif T.

11. The polynucleotide of claim 1, encoding a protein that contains Motif 1 and Motif 2.

12. The polynucleotide of claim 1, encoding a protein that contains Motif A, Motif B, Motif C, and Motif D.

13. The polynucleotide of claim 1, encoding a protein that contains at least two of said motifs.

14. The polynucleotide of claim 1, encoding a protein that contains at least four of said motifs.

15. The polynucleotide of claim 1, encoding a protein that contains all of said motifs.

16. The polynucleotide of claim 15, wherein the motifs occur in the order indicated in claim 1.

17. The polynucleotide of claim 1, which hybridizes to a nucleic acid having the mTERT cDNA sequence in SEQ ID NO:1 at 5° C. below T_m in 1 M sodium ion concentration, wherein T_m is the melting temperature under the same conditions of said nuleic acid hybridized to a complementary polynucleotide.

18. An isolated, purified or recombinant polynucleotide encoding a protein that contains SEQ. ID NO:2, or a fragment thereof that has telomerase reverse transcriptase activity when associated with telomerase RNA component.

19. A method of producing a telomerase protein, comprising expressing the polynucleotide of claim 1 in a host cell.

20. A method of producing a telomerase protein, comprising expressing the polynucleotide of claim 17 in a host cell.

21. A method of producing a telomerase protein, comprising expressing the polynucleotide of claim 18 in a host cell.

* * * * *

US006777203B1

(12) **United States Patent**
Morin et al.

(10) **Patent No.: US 6,777,203 B1**
(45) **Date of Patent: *Aug. 17, 2004**

(54) **TELOMERASE PROMOTER DRIVING EXPRESSION OF THERAPEUTIC GENE SEQUENCES**

(75) Inventors: **Gregg B. Morin**, Oakville (CA); **Serge P. Lichtsteiner**, Encinitas, CA (US); **Alain P. Vasserot**, Carlsbad, CA (US); **Robert R. Adams**, Redwood City, CA (US); **William H. Andrews**, Reno, NV (US)

(73) Assignee: **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/244,438**

(22) Filed: **Feb. 4, 1999**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/974,584, filed on Nov. 19, 1997, and a continuation-in-part of application No. 08/974,549, filed on Nov. 19, 1997, now Pat. No. 6,166,178.

(51) **Int. Cl.**[7] C12P 21/06; C12N 15/00

(52) **U.S. Cl.** 435/69.1; 435/455; 435/6; 435/320.1; 536/24.1

(58) **Field of Search** 435/320.1, 455, 435/69.1; 536/24.1

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,416,017	A	5/1995	Burton et al.	435/240.2
5,631,236	A	5/1997	Woo et al.	514/44
5,728,379	A	3/1998	Martuza et al.	424/93.2
5,907,083	A	5/1999	Robert et al.	800/205
5,998,205	A	12/1999	Hallenbeck et al.	435/325
6,054,575	A	4/2000	Villeponteau et al.	536/24.31
6,093,809	A	7/2000	Cech et al.	536/23.5
6,166,178	A *	12/2000	Cech	530/324
6,228,643	B1	5/2001	Greenland et al.	435/419
6,274,790	B1	8/2001	Kunst et al.	800/287
6,281,409	B1	8/2001	Woodhead et al.	800/287
6,300,095	B1	10/2001	Barredo Fuente et al.	435/69.1
6,306,656	B1	10/2001	Liu et al.	435/419
6,331,527	B1	12/2001	Parmacek et al.	514/44
6,610,839	B1	8/2003	Morin et al.	536/24.1

FOREIGN PATENT DOCUMENTS

GB	2317891	4/1998
WO	WO 98/07838	2/1998
WO	WO 98/14592	4/1998
WO	WO 98/14593	4/1998
WO	WO 98/21343	5/1998
WO	WO 98/37181	8/1998
WO	WO 99/01560	1/1999
WO	WO 99/33998	7/1999
WO	WO 99/38964	8/1999
WO	WO 00/46355	8/2000

OTHER PUBLICATIONS

Cong et al (Hum. Mol. Genet. 8(1): 137–142, 1999).*

Majumdar et al. The telomerase reverse transcriptase promoter drives efficacious tumor suicide gene therapy while preventing hepatotoxicity encountered with constitutive promoters. Gene Therapy 8:568, 2001.

Koga et al. A novel telomerase–specific gene therapy: Gene transfer of caspase–8 utilizing the human telomerase catalytic subunit gene promoter. Hu. Gene Ther. 11:1397, 2000.

Gu et al. Tumor–specific transgene expression from the human telomerase reverse transcriptase promoter enables targeting of the therapeutic effects of the Bax gene to cancers. Cancer Res. 60:5339, 2000.

Komata et al. Treatment of malignant glioma cells with the transfer of constitutively active Caspase–6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. Cancer Res. 61:5796, 2001.

Geron Corporation Press Release.. Geron Corporation and Genetic tTherapy, Inc. partner to develop cancer therapy. Jan. 7, 2002.

Berenstein, M., et al., "Different efficacy of in vivo herpes simplex virus thymidine kinase gene transduction and ganciclovir treatment on the inhibition of tumor growth of murine and human melanoma cells and rat glioblastoma cells", *Cancer Gene Therapy*, 6(4):358–366 (1999).

Bi, W., et al., "An HSV tk–mediated local and distant antitumor bystander effect in tumors of head and neck origin in athymic mice", *Cancer Gene Therapy*, 4(4):246–252 (1997).

Brand, K., et al., "Tumor cell–specific transgene expression prevents liver toxicity of the adeno–HSVtk/GCV approach", *Gene Therapy*, 5:1363–1371 (1998).

Cong, YS., et al., "The Human Telomerase Catalytic Subunit hTERT: Organization of the Gene and Characterization of the Promoter", *Human Molecular Genetics*, 8(1):137–142 (1999).

(List continued on next page.)

Primary Examiner—Dave T. Nguyen
Assistant Examiner—Richard Schnizer
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp

(57) **ABSTRACT**

The present invention is related to novel nucleic acids comprising telomerase reverse transcriptase (TERT) cis-acting transcriptional control sequences, including TERT human and mouse promoter sequences. The present invention is further directed to methods of using these cis-acting transcriptional control sequences, for example, to drive heterologous gene sequences; to modulate the level of transcription of TERT or to isolate novel trans-acting regulatory factors which bind to and modulate the activity of a TERT promoter.

23 Claims, 13 Drawing Sheets

-continued

<400> SEQUENCE: 22

actccagcat aatcttctgc ttccatttct tctcttccct cttttaaaat tgtgttttct 60

atgttggctt ctctgcagag aaccagtgta agctacaact taacttttgt tggaacaaat 120

tttccaaacc gccccttgc cctagtggca gagacaattc acaaacacag ccctttaaaa 180

aggcttaggg atcactaagg ggatttctag aagagcgacc cgtaatccta agtatttaca 240

agacgaggct aacctccagc gagcgtgaca gcccagggag ggtgcgaggc ctgttcaaat 300

gctagctcca taaataaagc aatttcctcc ggcagtttct gaaagtagga aaggttacat 360

ttaaggttgc gtttgttagc atttcagtgt ttgccgacct cagctacagc atccctgcaa 420

ggcctcggga gacccagaag tttctcgccc cttagatcca aacttgagca acccggagtc 480

tggattcctg ggaagtc 497

<210> SEQ ID NO 23
<211> LENGTH: 425
<212> TYPE: DNA
<213> ORGANISM: Mus sp.
<220> FEATURE:
<223> OTHER INFORMATION: Mouse TERT promoter

<400> SEQUENCE: 23

caagtgtgca ccaccatgcc ccgcgatatt cttatttttg agactgtttt ctatgctggt 60

ttctttgggg aactacacta aggtagcttc attgttggca taaatttctc agttcaggcc 120

catatctcct aagtagcaga actaagcaaa tctcaaacaa acccctcaaa aagactgatg 180

tccactaaac ggacttctaa aatagctcct gtaatcctga gcatttacaa ggcggcagac 240

ctcctataag ggagtaaata tgaaaacgcg cctgttcaaa tgctaggtcg gtggatagaa 300

gcaatttcct cagaaagctg aaggcaccaa aggttatatt tgttagcatt tcagtgtttg 360

ccaaactcag ctacagtaga gatcacagat tccctatttc ccagagattc aaaattcagc 420

agccc 425

What is claimed is:

1. A polynucleotide in which a promoter is operably linked to a heterologous encoding region,

wherein the promoter contains a nucleotide sequence that is least 90% identical to the sequence from position −117 to position −36 from the translation initiation site (position 13545) of SEQ. ID NO:1,

and wherein the promoter causes the encoding region to be transcribed preferentially in human cells that endogenously express telomerase reverse transcriptase (TERT) compared with human cells that do not endogenously express TERT.

2. The polynucleotide of claim 1, wherein the promoter contains a nucleotide sequence that is at least 80% identical to the sequence from position −239 to position −36 from the translation initiation site of SEQ. ID NO:1.

3. The polynucleotide of claim 1, wherein the promoter contains a nucleotide sequence that is at least 95% identical to the sequence from position −239 to position −36 from the translation initiation site of SEQ. ID NO:1.

4. The polynucleotide of claim 1, wherein the promoter contains the sequence from position −117 to position −36 from the translation initiation site of SEQ. ID NO:1.

5. The polynucleotide of claim 1, wherein the promoter contains the sequence from position −239 to position +1 from the translation initiation site of SEQ. ID NO:1.

6. The polynucleotide of claim 1, wherein the promoter is between about 400 to 900 nucleotides in length.

7. The polynucleotide of claim 1, wherein the promoter is between about 200 to 400 nucleotides in length.

8. The polynucleotide of claim 1, wherein the promoter is between about 100 to 200 nucleotides in length.

9. The polynucleotide of claim 1, wherein the encoding region encodes human telomerase reverse transcriptase.

10. The polynucleotide of claim 1, wherein the encoding region encodes a reporter protein detectable by fluorescence, phosphorescence, or enzymatic activity.

11. The polynucleotide of claim 10, wherein the reporter protein is selected from luciferase, glucuronidase, chloramphenicol acetyl transferase, green fluorescent protein, alkaline phosphatase, and galactosidase.

12. The polynucleotide of claim 1, wherein said heterologous encoding region encodes a product that is toxic to the cell or renders the cell more susceptible to toxicity of a drug.

13. The polynucleotide of claim 12, wherein the encoding region encodes a protein selected from ricin, diphtheria toxin, other polypeptide toxins, thymidine kinase, and an enzyme that induces apoptosis.

14. The polynucleotide of claim 12, wherein the drug is ganciclovir.

15. A viral vector comprising the polynucleotide of claim 1.

16. The vector of claim 15, which is an adenovirus vector.

17. A mammalian cell comprising the polynucleotide of claim 1.

18. A method of expressing an encoding region in a cell, comprising contacting the cell in vitro with the polynucleotide of claim 1.

19. A method of killing a mammalian cell that expresses TERT, comprising expressing the polynucleotide of claim 12 in the cell in vitro, wherein said heterologous encoding region encodes a product that is toxic to the cell.

20. The method of claim 19, wherein the cell that expresses TERT is a cancer cell.

21. A method of screening a compound that modulates expression of telomerase reverse transcriptase (TERT), comprising contacting a cell transfected with a polynucleotide according to claim 10 with the compound in vitro, and correlating any resulting change in expression of the reporter protein with an ability of the compound to modulate TERT expression.

22. A method of producing a protein, comprising expressing a polynucleotide according to claim 1 in a cell in vitro, wherein said heterologous encoding region encodes the protein.

23. A method of killing a mammalian cell that expresses TERT, comprising expressing the polynucleotide of claim 12 in the cell in vitro, wherein said heterologous encoding region encodes a product that makes the cell more susceptible to toxicity of said drug.

* * * * *

US006440735B1

(12) **United States Patent**
Gaeta

(10) **Patent No.: US 6,440,735 B1**
(45) **Date of Patent: Aug. 27, 2002**

(54) **DENDRITIC CELL VACCINE CONTAINING TELOMERASE REVERSE TRANSCRIPTASE FOR THE TREAMENT OF CANCER**

(75) Inventor: **Federico C. A. Gaeta**, Mountain View, CA (US)

(73) Assignee: **Geron Corporation**, Menlo Park, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/675,321**

(22) Filed: **Sep. 28, 2000**

Related U.S. Application Data

(63) Continuation of application No. PCT/US99/06898, filed on Mar. 30, 1999.
(60) Provisional application No. 60/112,006, filed on Mar. 31, 1998.

(51) **Int. Cl.**[7] C12N 5/08; A01N 63/00; A61K 48/00

(52) **U.S. Cl.** 435/372.2; 435/372.3; 424/93.21

(58) **Field of Search** 435/372.2, 372.3; 424/93.21

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,839,290	A	6/1989	Kaieda et al.	435/240.23
5,583,016	A	12/1996	Villeponteau et al.	435/91.3
5,645,986	A *	7/1997	West et al.	435/6
5,648,219	A	7/1997	MacKay et al.	435/6
5,656,638	A *	8/1997	Gaeta et al.	514/301
5,747,317	A	5/1998	Cao	435/194
5,770,422	A	6/1998	Collins	435/194
5,858,777	A	1/1999	Villeponteau et al.	435/325
5,866,115	A	2/1999	Kanz et al.	424/93.7
5,871,728	A	2/1999	Thomson et al.	424/93.7
5,874,307	A	2/1999	Ohno et al.	435/372.3
5,917,025	A	6/1999	Collins	536/23.2
5,962,318	A	10/1999	Rooney et al.	435/325
5,962,320	A *	10/1999	Robinson	435/366
5,968,506	A *	10/1999	Weinrich et al.	424/94.5
5,972,627	A	10/1999	Galy	435/7.21
5,981,707	A	11/1999	Harrington et al.	530/350
5,994,126	A	11/1999	Steinman et al.	435/325
6,004,807	A	12/1999	Banchereau et al.	435/325
6,008,004	A	12/1999	Olweus et al.	435/7.24
6,010,905	A	1/2000	Cohen et al.	435/372
6,015,554	A	1/2000	Galy	424/93.7
6,017,527	A	1/2000	Maraskovsky et al.	424/93.71
6,033,669	A	3/2000	Jondal	424/193.1
6,077,519	A	6/2000	Storkus et al.	424/277.1
6,080,409	A	6/2000	Laus et al.	424/192.1
6,093,809	A	7/2000	Cech et al.	536/23.5
6,166,178	A *	12/2000	Cech et al.	530/324
6,224,870	B1 *	5/2001	Segal	424/192.1
6,261,836	B1 *	7/2001	Cech et al.	435/325
6,277,613	B1	8/2001	De Lange et al.	435/193

FOREIGN PATENT DOCUMENTS

CH	689672 A9	2/2000
WO	WO 93/20185	10/1993
WO	WO 94/02156	2/1994
WO	WO 94/21287	9/1994
WO	WO 94/28113	12/1994
WO	WO 95/34638	12/1995
WO	WO 96/23060	8/1996
WO	WO 97/04802	2/1997
WO	WO 97/07200	2/1997
WO	WO 97/22349	6/1997
WO	WO 97/24447	7/1997
WO	WO 97/29182	8/1997
WO	WO 97/29183	8/1997
WO	WO 97/40182	10/1997

(List continued on next page.)

OTHER PUBLICATIONS

Nakamura et al., Science 277 (5328), 955–959 (Aug. 15, 1997).*
Banchereau, J., et al., "Dendritic Cells and the Control of Immunity", Nature, 392:245–252 (1998).
Banchereau, J., et al., "Immunobiology of Dendritic Cells", Annu. Rev. Immunol., 18:767–811 (2000).
Cong, YS., et al., "The Human Telomerase Catalytic Subunit hTERT: Organization of the Gene and Characterization of the Promoter", *Human Molecular Genetics*, 8(1):137–142 (1999).
Greaves, M., Is Telomerase Activity in Cancer Due to Selection of Stem Cells and Differentiation Arrest?, Trends Genet., 12(4):127–128 (1996).
Greenberg, R.A., et al., "Telomerase reverse transcriptase gene is a direct target of c–Myc but is not functionally equivalent in cellular transformation", *Oncogene*, 18:1219–1226 (1999).
Greener, M., "Telomerase: The Search for a Universal Cancer Vaccine", Molecular Med. Today, 6:257 (2000).
Hart, D., "Dendritic Cells: Unique Leukocyte Populations Which Control the Primary Immune Response", Blood, 90(9):3245–3287 (1997).
Hsu, et al., "Vaccination of Patients With B–Cell Lymphoma Using Autologous Antigen–Pulsed Dendritic Cells", Nature Med., 2:52–58 (1996).

(List continued on next page.)

Primary Examiner—Kenneth R. Horlick
(74) *Attorney, Agent, or Firm*—J. Michael Schiff; David J. Earp

(57) **ABSTRACT**

The invention provides a method of activating a T lymphocyte by contacting the T lymphocyte with a dendritic cell (DC) that presents a telomerase reverse transcriptase (TRT) peptide in the context of a MHC class I or MHC class II molecule. The DC may be pulsed with a TRT polypeptide or may comprise a recombinant polynucleotide encoding a TRT such as hTRT. The invention also provides DCs comprising a recombinant TRT polynucleotide. The methods and compositions of the invention are used in prevention and treatment of cancers and other cell proliferation diseases or conditions.

22 Claims, 2 Drawing Sheets

-continued

```
Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

What is claimed is:

1. A composition comprising antigen-presenting cells containing a polypeptide that comprises at least 6 consecutive amino acids of telomerase reverse transcriptase (TRT; SEQ. ID NO:2), and a pharmaceutical carrier suitable for human administration; whereupon administration of the composition to a human subject induces an anti-TRT immunological response.

2. The composition of claim 1, wherein the antigen-presenting cells are dendritic cells.

3. The composition of claim 1, wherein the antigen-presenting cells have either been pulsed ex vivo with a polypeptide containing said consecutive amino acids, or modified ex vivo with a polynucleotide encoding said consecutive amino acids.

4. The composition of claim 1, wherein the polypeptide comprises at least 8 consecutive amino acids of SEQ. ID NO:2.

5. The composition of claim 1, further comprising a cytokine.

6. The composition of claim 5, wherein the cytokine is GM-CSF or IL-2.

7. The composition of claim 1, wherein the immunological response comprises both TRT-specific antibody and TRT-specific cytotoxic T cells.

8. A method for preparing the composition of claim 1, comprising isolating mononuclear leukocytes from peripheral blood, optionally fractionating or differentiating the leukocytes, and then either:

a) pulsing the leukocytes with a polypeptide containing said consecutive amino acids; or

b) modifying the leukocytes with a polynucleotide encoding said consecutive amino acids.

9. The method of claim 8, wherein the leukocytes are pulsed with a polypeptide containing 8–12 consecutive amino acids of SEQ. ID NO:2.

10. The method of claim 8, wherein the leukocytes are modified with a polynucleotide encoding at last 12 consecutive amino acids of SEQ. ID NO:2.

11. A method for eliciting an anti-TRT immunological response in a subject, comprising administering to the subject the composition of claim 1.

12. The composition of claim 1, wherein the antigen-presenting cells contain a plurally of such polypeptides.

13. A method for preparing cytotoxic T cells specific for telomerase reverse transcriptase (TRT), comprising combining T lymphocytes ex vivo with antigen-presenting cells containing a polypeptide that comprises at least 6 consecutive amino acids of telomerase reverse transcriptase (TRT; SEQ. ID NO:2), so as to cause T lymphocytes specific for TRT to proliferate.

14. The method of claim 13, wherein the antigen-presenting cells are dendritic cells.

15. The method of claim 13, wherein the antigen-presenting cells have been pulsed ex vivo with the polypeptide.

16. The method of claim 13, wherein the antigen-presenting cells have been modified with a polynucleotide ex vivo so as to express the polypeptide.

17. A cytotoxic T cell produced according to the method of claim 13.

18. A method for providing a subject with T cell immunity against target cells bearing TRT antigenic peptides, comprising administering to the subject cytotoxic T cells according to claim 17.

19. An isolated cytotoxic T cell specific for telomerase reverse transcriptase (TRT).

20. The cytotoxic T cell of claim 19, which is a CD8+ Class-I restricted T cell.

21. A pharmaceutical composition comprising a plurality of cytotoxic T cells according to claim 19 in a pharmaceutically acceptable carrier suitable for human administration.

22. A method for providing a subject with T cell immunity against target cells bearing TRT antigenic peptides, comprising administering to the subject cytotoxic T cells according to claim 19.

* * * * *